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Identification of time-to-peak on dynamic 18F-FET-PET as a prognostic marker specifically in IDH1/2 mutant diffuse astrocytoma

Suchorska, Bogdana ; Giese, Armin ; Biczok, Annamaria ; Unterrainer, Marcus ; Weller, Michael ; Drexler, Mark ; Bartenstein, Peter ; Schüller, Ulrich ; Tonn, Jörg-Christian ; Albert, Nathalie L

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Neuro-Oncology

Identification of time-to-peak on dynamic 18F-FET-PET as a prognostic marker specifically in IDH1/2 mutant diffuse astrocytoma

--Manuscript Draft--

Manuscript Number:	N-O-D-17-00290R1
Full Title:	Identification of time-to-peak on dynamic 18F-FET-PET as a prognostic marker specifically in IDH1/2 mutant diffuse astrocytoma
Article Type:	Clinical Investigations
Keywords:	glioma, IDH1/2 mutation, 1p/19q co-deletion,18F-FET-PET, kinetic analysis
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Abstract:	Background: Stratification of glioma according to isocitrate dehydrogenase 1/2 (IDH1/2) mutation and 1p/19q co-deletion status has gained major importance in the new WHO classification. Parameters derived from 18F-FET-PET uptake dynamics such as minimal time-to-peak (TTPmin) allow discrimination between different prognostic glioma subgroups, too. The present study aimed at exploring whether TTPmin analysis provides prognostic information beyond the WHO classification. Methods: Three-hundred patients with newly diagnosed WHO 2007 grade II-IV gliomas with 18F-FET-PET imaging at diagnosis were grouped into 4 subgroups (IDH1/2 mut/1p/19q co-del; IDH1/2 mut/1p/19q non co-del, IDH1/2 wildtype WHO grade II and III tumors, and glioblastoma). Clinical and imaging factors such as age, Karnofsky performance score, treatment, TTPmin and maximal tumor-to-brain ratio (TBRmax) were analyzed with regard to progression-free and overall survival (PFS and OS) via univariate and multivariate regression analysis. Results: PFS and OS were longest in the IDH1/2 mut/1p/19q co-del subgroup followed by IDH1/2 mut/1p/19q non co-del, IDH1/2 wt patients and GBM ($p<0.001$). Further, outcome stratified by TTPmin with a cutoff of 17.5 minutes revealed significantly longer PFS and OS in patients with TTPmin >17.5 minutes ($p<0.001$ for PFS and OS). Lower

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	TBRmax values or the absence of 18F-FET-uptake were also associated with favorable outcome in the entire group. In the subgroup analyses, longer median TTPmin was associated with improved outcome specifically in the IDH1/2 mut/1p/19q non co-del group. Conclusion: 18F-FET-PET-derived dynamic analysis defines prognostically distinct sub-groups of IDH1/2 mutant/ 1p/19q-non-co-deleted gliomas which cannot be distinguished as yet by molecular marker analysis.

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Cover Letter

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NEUROCHIRURGISCHE KLINIK UND POLIKLINIK

DIREKTOR:
PROF. DR. JÖRG-CHRISTIAN TONN

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To
Patrick Y. Wen
Senior Editor
Neuro-oncology

Dear Professor Wen,

dear Ladies and Gentlemen of the editorial board,

Please find enclosed our revised manuscript "**Identification of time-to-peak on dynamic ^{18}F-FET-PET as a prognostic marker specifically in *IDH1/2* mutant diffuse astrocytoma**" **(Ms. No. N-O-D-17-00290R1).**

We have considered the reviewers' comments and have revised the manuscript accordingly, adding an extensive amount of MRI data as well as a more refined molecular/histological evaluation. We think that the manuscript has improved substantially and hope it is now suitable for publication in *Neuro-Oncology*.

We agree to pay the fee for color figures.

Kind regards,

Bogdana Suchorska

(Correspondent author)

Jörg-Christian Tonn

(Senior author)

Dear Dr. Wen,

Please find enclosed the revised version of our manuscript entitled **"Identification of time-to-peak on dynamic ^{18}F-FET-PET as a prognostic marker specifically in IDH1/2 mutant diffuse astrocytoma"** which we hope is now suitable for publication in Neuro-Oncology.

We have considered the suggestions of the reviewers and have revised our manuscript substantially according to their recommendations, especially regarding the molecular subgroup classification and the addition of MRI-based tumor parameters. We believe that these changes have helped to emphasize our hypothesis concerning the relevance of ^{18}F-FET-PET based TTP_{min} for outcome in glioma and to improve the readability of the manuscript.

Kind regards

Bogdana Suchorska

Correspondent author

Reviewer #1: Review of Ms. Ref. No.: N-O-D-17-00290

Title: Identification of time-to-peak on dynamic 18F-FET-PET as a prognostic marker

specifically in IDH1/2 mutant diffuse astrocytoma

Author: Suchorska et al.

General comments:

PET using radiolabeled amino acids is a very promising diagnostic tool for the management of cerebral gliomas. The Response Assessment in Neuro-Oncology working group has recently recommended the use of amino acid PET imaging for brain tumour management in addition to MRI (Albert et al., 2016). The main advantages of amino acid PET are the definition of tumor extent, diagnosis of tumor recurrences and treatment monitoring. Tumor grading and prognostication by amino acid PET may be of additional value but so far has only limited clinical relevance (Langen et al., 2017). In the present study, Suchorska et al. demonstrate that PET using the amino acid tracer F-18-Fluoroethyltyrosine may be helpful to provide prognostic information beyond histopathological and molecular markers especially in the subgroup of gliomas with IDH1/2 mutation and intact 1p/19q. The study is highly relevant but some aspects concerning data evaluation and methodology need to be revised.

Special comments:

Material and Methods/18F-FET-PET and MR imaging/line 10

"Tumors were termed 18F-FET-"positive" when TBRmax was above 1.6 (Ref 12)."

Comment: Ref. 12 deals with brain metastasis. Please comment.

RESPONSE

We thank the reviewer for this comment and apologize: this was an error. We have changed the mentioned reference for the two following references: (11) Unterrainer M: Serial FET PET imaging of primarly ^{18}F-FET-negative glioma: does it make sense. J Nucl Med. 2016 Aug. 57 (12): 1177-82. Floeth FW: Prognostic value of 18F-fluoroethyl-L-tyrosine PET and MRI in small nonspecific incidental brain lesions. J Nucl Med. 2008 May; 49(5):730-7.

Material and Methods/18F-FET-PET and MR imaging/third paragraph

"As explained in detail previously (Ref 8), TTP was identified for each slice and the shortest TTP being present in at least two adjacent slices was defined as minimal TTP (TTPmin)."

Comment: With respect to the fact that tumor tissue specimen were obtained from the area with highest FET uptake (see next paragraph/surgical procedure) it would be important to know in how many cases the shortest TTP was found in tumor areas outside the maximum FET uptake value for the whole tumor. This has possibly consequences for biopsy guidance.

RESPONSE

In this study, TTP_{min} analyses were performed quantitatively without spatial correlation of TTP_{min} and uptake intensity on static images. As TTP_{min} was not analyzed in a voxel-wise manner (slice-by-slice analysis within a 90% iso-contour ROI), a proper spatial correlation is not feasible. However, we also noticed that the slices with minimal TTP did not necessarily correspond to the slice with maximum ^{18}F-FET uptake. This was observed in around one third of the cases and might indeed serve as guidance for biopsies in the future.

Material and Methods/Histopathology and molecular genetic markers, page 5

"Histological diagnosis was performed by experienced neuropathologists (U.S., A.G.) according to the WHO classification version 2007 blinded for PET and MRI findings."

Comment: With respect to the fact that molecular parameters were available for nearly all patients it is difficult to understand that the classification was not carried out according to the WHO classification of 2016. It would significantly increase the meaningfulness of the study if the presentation of the data (Table 1, suppl. Table 1 and 2, suppl. Figure 2) and univariate/multivariate analysis (table 3 and 4) would be properly adapted to the WHO classification of 2016.

RESPONSE

The revision of the WHO classification, as far as gliomas of adulthood are concerned, has indeed introduced the molecular markers that we used in our study. Regarding the single parameters shown in figures and tables mentioned by the reviewer, none changes in this context; e.g., a diffuse astrocytoma with the respective morphological picture has still to be classified as WHO grade II, even if it does not harbor an *IDH* mutation. In the entire paper, we never included any "old" histological entities, such as oligoastrocytoma in our calculations. However, in accordance with this helpful comment we decided to show glioblastoma (being a separate entity in the WHO classification) as an extra group and have revised all calculations, tables and figures accordingly.

Results:

Comment: It would be helpful for future meta-analysis if a supplementary table with detailed data for each patient would be provided.

RESPONSE

We now have provided a respective datasheet as Supplemental Table 2.

Discussion:

Comment: Please add a statement that TBRmax values are dependent on scanner resolution and data processing and may not be comparable between different centers. The same concerns the determination of TTP.

RESPONSE

We agree and have added a respective paragraph within the discussion section of the study (p. 14,ll. 23-24 and p.15, ll.1-2).

References

Albert NL, Weller M, Suchorska B, Galldiks N, Soffietti R, Kim MM, la Fougere C, Pope W, Law I, Arbizu J, Chamberlain MC, Vogelbaum M, Ellingson BM, Tonn JC (2016) Response Assessment in Neuro-Oncology working group and European Association for Neuro-Oncology recommendations for the clinical use of PET imaging in gliomas. Neuro-oncology 18:1199-1208.

Langen KJ, Galldiks N, Hattingen E, Shah NJ (2017) Advances in neuro-oncology imaging. Nature reviews Neurology.

RESPONSE

We have incorporated the two references mentioned by the reviewers into the manuscript (Citation number 22 and 27).

Reviewer #2: The authors correlate molecular characteristics of gliomas (IDH1/2 status, 1p/19q co-deletion with parameters) derived from 18F FET PET (minimal time to peak, TTPmin; maximal tumor-to-brain ratio, TBR max), grouping these patients into 3 groups (IDH1/2 wildtype; IDH1/2 mutated and codeleted; and IDH1/2 mutated and non-codeletion).

They find, as expected, that the prognosis for patients (PFS, OS) was best in patients IDH1/2 mutated and codeleted tumors, followed by IDH mutated, non-codeleted tumors. Overall, PFS/OS were more favorable in patients with time TTPmin >17.5 minutes, as well as low TBRmax values. In the analysis of molecular subgroups TTPmin was associated with improved outcome specifically in the IDH1/2 mut/1p/19q non co-del group, but neither in the IDH1/2 mut/1p/19q co-del nor in the IDH 1/2 wt group. One interesting finding was that TTPmin was stronger than grade II or III histology in predicting outcome in non-codeleted IDH mutated tumors.

This study was obviously designed to predict molecular pathology from FET PET.

RESPONSE

In fact, the present study was not primarily designed to predict molecular pathology from ^{18}F-FET-PET as *IDH 1/2* mutation and co-deletion 1p/19q analysis are widely available. The aim of this study was rather to analyze whether ^{18}F-FET-PET parameters are associated with prognosis beyond the prognostic value of the molecular markers *IDH 1/2* mutation and 1p/19q co-deletion.

This study confirms the value of TTPmin and TBRmax as an indicator of prognosis, which is not new. The observation of a predictive value of TTPmin in IDH1 non-codeleted tumors is and might affect treatment decisions. Furthermore, this observation indicates possible unknown factors that affect TTPmin govern survival rather than conventional grading.

There are some weaknesses. This is a retrospective study in which patients were accrued between 2004 and 2014. The authors do not indicate how many other variables related to FET PET were tested before identifying TTPmin and TBRmax to discriminate prognostic subgroups. Such multiple testing would have to be accounted for statistically.

RESPONSE

This study was designed to test the two ^{18}F-FET-PET derived parameters TBR_{max} versus TTP_{min} in their explanatory power in light of the molecular markers *IDH 1/2* mutation and 1p/19q co-deletion, as TBR_{max} is still the widely available parameter in most centers. However, based on the results published by our group concerning TTP_{min} and its prognostic value within different glioma subgroups, our hypothesis for the present paper was that TTP_{min} would prove superior to the standard acquisition reflected by TBR_{max}. As far as biological tumor volume (BTV) is concerned, we did not take it into account because it is dependent on the threshold value set for the definition of the volume of interest. However, so far no standard has been agreed upon for appropriate common TBR-threshold for volume definition for either type of glioma (see Albert NL, Weller M, Suchorska B, Galldiks N, Soffietti R, Kim MM, la Fougere C, Pope W, Law I, Arbizu J, Chamberlain MC, Vogelbaum M, Ellingson BM, Tonn JC (2016) Response Assessment in Neuro-Oncology working group and European

Association for Neuro-Oncology recommendations for the clinical use of PET imaging in gliomas. Neuro-oncology 18:1199-1208).

My strongest worry is, however, that the authors have omitted all conventional MR imaging parameters in their multivariate analysis, foremost contrast enhancement, and tumor size which are known prognostic factors. The authors speculated that perfusion might influence apparent FET PET dynamics. Blood-brain barrier disruption or increased CBF might equally play a role, and much of this is reflected by simple enhancement on the MRI. The authors should include variables related to MRI contrast-enhancement in their model. I would be tempted to say, that, by doing so, significance would be lost. Even the interesting observation of TTPmin being more strongly prognostic than WHO grade in the IDH mutated non-codeleted tumor suffers as long as MRI data are missing adjusting for size and enhancement.

RESPONSE

We thank for this valuable suggestion. Accordingly, we have now included information on tumor location, tumor size and absence or presence of contrast enhancement (CE) into our data presentation (Table 1), as well as presence of CE into both univariate and multivariate analyses (Table 3). A statement concerning differences in tumor size and presence of CE has been added into the results section (p. 8, line 15-19).

While CE is a powerful discriminator in the univariate analyses, it loses its significance in the multivariate analysis whereas TTP_{min} remains an independent prognosticator for OS, independent of *IDH1/2* mutation and WHO grading.

Another worry is that the collective of patients might be highly selected. Only 62 of 300, that is 20% received surgery, which is not standard of care according to various neuro-oncological guidelines, and would not be expected for the neurosurgical centers involved in this study. This observation insinuates that we here might be seeing a subgroup with diffuse, "non-resectable" tumors. Again no MRI descriptors are presented (enhancement, size, edema, eloquent locaton etc.) to counter this concern. The authors should comment on this.

RESPONSE

As a referral center for stereotactic biopsies, we have a relatively high proportion of patients undergoing stereotactic biopsy either due to tumor location, patient's age or preference.

Especially in WHO II grade glioma, surgery is mostly offered in well-delineated, circumscribed tumors, where the value of surgical resection is likely. Many diffuse, poorly delineable tumors not qualifying for resection do not appear in most surgical series but were included in the present study: 170 patients were classified as eloquent within our cohort as opposed to 130 non-eloquent cases. Information on tumor location, size, eloquent location and contrast enhancement can now be found in Table 1.

Along these lines it is worrisome that surgical procedure (biopsy vs. resection) does not appear to be prognostic, not even in the univariate analysis. This contradicts the general understanding of the value of surgery in low and high grade gliomas patients. How can this be explained?

RESPONSE

Patients with gliomas of WHO grades III and IV received surgery more often than those with WHO II tumors (47/62 surgeries in WHO III/IV versus only 15/62 in WHO II). We deliberately pooled all patients across WHO grades II – IV for analysis, and surgery showed no association with outcome in univariate testing.

Finally, I wonder whether the morphology of FET uptake might be related to prognosis. The authors do not differentiate between focal uptake of tracer (in hotspots) or uptake by the entire tumor.

RESPONSE

It is difficult to evaluate objectively whether the uptake is found in the entire tumor or in only parts of the tumor, as the "real" extent of the entire tumor depends on the threshold set for volume definition. Thus, the more objective way of evaluating mere visual appearance of ^{18}F-FET uptake based on current literature (Ewelt et al. Finding the anaplastic focus in diffuse gliomas: The value of Gd-DTPA enhanced MRI, FET-PET, and intraoperative, ALA-derived tissue fluorescence Clinical Neurology and Neurosurgery; Unterrainer et al Serial ^{18}F-FET PET Imaging of Primarily ^{18}F-FET-Negative Glioma: Does It Make Sense?, J Nucl. Med. 2017) is a qualitative description in terms of "^{18}FET-negative" vs. "^{18}FET-positive" glioma in analogy to the evaluations of MR parameters in clinical trials, which also use binary classification into contrast-enhancing vs. non-enhancing glioma.

Reviewer #3: General Comments:

This retrospective study evaluating the value of dynamic 18F-FET PET in 300 patients with newly diagnosed gliomas is well designed (especially for a retrospective study) and implemented. The authors have an amazing data base of imaging and clinical data to correlate with recently update WHO guidelines in brain tumors. The correlations between imaging data, histopathology, WHO grade, and clinical outcomes are very informative in most sub-types of tumors and this new information has great potential to provide clinically useful information for individual patients. This reviewer feels the authors have been very thoughtful and somewhat conservative in the discussion and interpretation of their findings, which bodes well for similar future studies.

This reviewer finds no major methodological flaws, though has a number of points for consideration for minor revision or clarification.

Specific Comments:

Materials and Methods Section:

1. When defining the regions of interest (ROI's), can you better explain how the ROI's were obtained? Assuming this was an automated process, please briefly state the methods/software used

and how individual tumors borders/ROI's may have been confirmed and/or adjusted if needed (i.e. did the 90% iso-contour overlap with adjacent scalp activity or other non-specific uptake and need to be manually adjusted? Was the uptake correlated with T1 or T2 MRI anatomic findings? Did this potentially affect TBRmax values, etc?)

RESPONSE

The ROIs were semi-automatically drawn using “PET Display Dynamic”, which is a software tool implemented in our HERMES work station. The ROIs did not need to be adjusted due to the high iso-contour threshold of 90%. As we did not evaluate the tumor volume in this study, the uptake was not spatially correlated with MRI findings. We changed the methods section according to the reviewer’s suggestion (p.5., ll. 17-22).

2. Can you provide more clarification as to how the TTP was identified and calculated (just briefly, versus long explanation as previously published).

RESPONSE

We thank the reviewer for the comment and have changed the methods section according to the reviewer’s suggestion (p. 5, method section).

3. The authors define "progression" by MRI imaging as (1) any new contrast enhancement… in a previously non-enhancing tumor or (2) T2 diameter of the tumor enlarged above 25%. If this definition was strictly applied to all patients in a post-treatment setting, there should be a fairly high number of patients that experienced pseudoprogression/treatment related change during the course of the study. Did the authors attempt to identify cases of pseudoprogression and exclude this time-point from inclusion into progression free survival (PFS) time? If yes, how many patients were affected and how were they appropriately excluded. If no, the authors could consider re-evaluating the data for this phenomena and re-analyzing to determine if PFS is significantly changed in the various sub-types if pseudoprogression is not considered true progression.

RESPONSE

We thank the reviewer for this valuable comment, as it is of course an important issue when analyzing PFS. We have, however, accounted for pseudo-progression when evaluating the data presented in the current study and have calculated the PFS with the date of real progression only. Out of the 178 patients who have experienced progression, 93 patients received stereotactic biopsy to rule out pseudo-progression. In the remaining 85 patients, 45 have died within 4 months after documentation of progression, 7 presented with a T_2 diameter enlargement > 25% without prior therapy, 13 patients had a new contrast enhancement in a previously non-enhancing tumor and 8 patients had a distant tumor progression. In the remaining 12 patients, MRI was repeated within 8 weeks to rule out pseudo-progression.

4. After surgical resection of tumors based on MRI and PET based neuronavigation, did any patients have 18F-FET PET scans shortly after resection to determine completeness of PET-avid tumor resection? If yes, is it possible and practical to determine the effect of completeness of

resection on PFS/OS of the various sub-types? This was not included as an aim of this study and does not need to be added, though if the data is readily available it may be of clinical interest.

RESPONSE

Although this is a highly interesting aspect, we have not addressed this issue mainly due to the fact that post-operative PET was obtained only in 34 patients.

Results Section:

1. Was there an attempt to correlate tumor SUVmax data as was done with TTP and TBR? Was the background 18F-FET uptake in the contralateral hemisphere fairly uniform across patients (and thus SUVmax and TBR may be strongly correlated) or was there significant heterogeneity in background uptake?

RESPONSE

To ensure intra- as well as inter-individual comparability of tumoral uptake intensity, it is recommended to evaluate the SUV_{max} as ratio to the healthy background activity. Consequently, SUV_{max} and TBR ($=SUV_{max}/SUV_{background}$) are strongly correlated, but are not correlated with the background activity, which does not only vary between patients, but even between scans at different time points within one patient. If background activity was equal in all patients, the calculation of a ratio would be dispensable.

2. Did the authors make an attempt to define more specific cut-off values for TTPmin and TBRmax versus choosing the median values? Although choosing median values is more straightforward and certainly does prove the authors point that there are differences in patients with low versus high values in many sub-types, perhaps there is a more statistically significant method for determining TTP and TBR values to show significant differences in PFS/OS.

RESPONSE

We thank the reviewer for this comment. We hypothesize that the reviewer refers to ROC analysis when suggesting other statistical methods, which we have considered, but did not provide for two reasons. The first argument against it is the one mentioned by the reviewer himself: there are far too many differences in TBR_{max} within the different subgroups. Furthermore, due to the method for obtaining TTP_{min} (averaged values obtained from different frames instead of continuously scaled values), ROC would not be possible to provide here. Thus, when performing ROC for TBR_{max} and median split for TTP_{min}, we would, in addition to the already complicated comparison of different histologies and molecular subgroups, add two different statistical methods for threshold definition, which would not improve readability of the paper.

Discussion Section:

1. Arguments appear to be well supported when possible and the authors do not appear to make any claims they cannot support with the data. No suggestions for change.

General Comments:

1. The tables and especially figures are very good. However, some can use a little more explanation in the key descriptions and at least one does not clearly define the abbreviations used.

We thank the reviewer for this comment and have provided a more appropriate legend description.

2. Thank you for submitting this very interesting study. Hopefully this PET technique will be used more widely throughout the world and use information from this study to help stratify patients into low- and high-risk groups.

N-O-D-17-00290R1

Identification of time-to-peak on dynamic ^{18}F-FET-PET as a prognostic marker specifically in *IDH1/2* mutant diffuse astrocytoma

Running title: TTP as prognostic marker in IDH mutant astrocytoma

Bogdana Suchorska[1,2], Armin Giese[2,3], Annamaria Biczok[1,2], Marcus Unterrainer[2,4], Michael Weller[5], Mark Drexler[2,4], Peter Bartenstein[2,4], Ulrich Schüller[2,3*], Jörg-Christian Tonn[1,2#] and Nathalie L. Albert[2,4#]

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Short title: ^{18}F-FET-PET, *IDH1/2* mutation and 1p/19q co-deletion in glioma

Word count: 4483, 3 Tables, 3 Figures, 5 Supplementary Files (3 Tables + 2 Figures)

Keywords: glioma, *IDH1/2* mutation, 1p/19q co-deletion,^{18}F-FET-PET, kinetic analysis, prognostic value

Funding:

We thank the Remark-Moolenaars family for a financial grant to support this study.

Abstract

Background: Stratification of glioma according to isocitrate dehydrogenase 1/2 (*IDH1/2)* mutation and 1p/19q co-deletion status has gained major importance in the new WHO classification. Parameters derived from ^{18}F-FET-PET uptake dynamics such as minimal time-to-peak (TTP_{min}) allow discrimination between different prognostic glioma subgroups, too. The present study aimed at exploring whether TTP_{min} analysis provides prognostic information beyond the WHO classification.

Methods: Three-hundred patients with newly diagnosed WHO 2007 grade II-IV gliomas with ^{18}F-FET-PET imaging at diagnosis were grouped into 4 subgroups (*IDH1/2* mut/1p/19q co-del; *IDH1/2* mut/1p/19q non co-del, *IDH1/2* wildtype WHO grade II and III tumors, and glioblastoma). Clinical and imaging factors such as age, Karnofsky performance score, treatment, TTP_{min} and maximal tumor-to-brain ratio (TBR_{max}) were analyzed with regard to progression-free and overall survival (PFS and OS) via univariate and multivariate regression analysis.

Results: PFS and OS were longest in the *IDH1/2* mut/1p/19q co-del subgroup followed by *IDH1/2* mut/1p/19q non co-del, *IDH1/2* wt patients and GBM ($p<0.001$). Further, outcome stratified by TTP_{min} with a cutoff of 17.5 minutes revealed significantly longer PFS and OS in patients with TTP_{min} >17.5 minutes ($p<0.001$ for PFS and OS). Lower TBR_{max} values or the absence of ^{18}F-FET-uptake were also associated with favorable outcome in the entire group. In the subgroup analyses, longer median TTP_{min} was associated with improved outcome specifically in the *IDH1/2* mut/1p/19q non co-del group.

Conclusion: ^{18}F-FET-PET-derived dynamic analysis defines prognostically distinct sub-groups of *IDH1/2* mutant/ 1p/19q-non-co-deleted gliomas which cannot be distinguished as yet by molecular marker analysis**.**

Importance of the study:

In light of the revised WHO 2016 classification, management of gliomas will increasingly depend on their molecular genetic profile. Data from more refined imaging techniques, such as the dynamic ^{18}F-FET-PET uptake analysis discussed in this manuscript, indicate that imaging biomarkers might provide additional information relevant for prognosis. The current manuscript explores the value of dynamic ^{18}F-FET-PET in 300 patients with a newly diagnosed WHO grade II-IV glioma; our data show dynamic uptake analysis reflected by time-to-peak (TTP) to provide further prognostic information within molecular subgroups according to *IDH1/2* mutation and co-deletion 1p/19q independently of WHO grading.

INTRODUCTION

Tailoring treatment options in glioma patients according to an individual risk profile is gaining increasing importance in the field of neuro-oncology[1]. Recent analyses of large cohort studies have uncovered a dominant association of molecular markers with clinical outcome in glioma patients[2-5]: As a consequence, the 2016 revision of

the WHO classification of tumors of the central nervous system accounts for molecular markers for sub-classification of gliomas[6]: *IDH1/2* mutant tumors have a different biology and better outcome than *IDH1/2* wildtype tumors, and *IDH1/2* mutant tumors are further subdivided into 1p/19q-co-deleted – associated with oligodendroglial morphology and better outcome – and non-co-deleted tumors – associated with astrocytic morphology and intermediate outcome.

Recent advances in molecular imaging via *O*-(2-^{18}F-fluorethyl)-L-tyrosine positron emission tomography (^{18}F-FET-PET) have led to the establishment of several non-invasive, imaging-derived prognostic factors such as biological tumor volume (BTV) and dynamic tracer uptake represented by time activity curves (TAC) and time-to-peak (TTP) evaluation[7-9]. In particular the dynamic evaluation of uptake including TTP analysis is highly associated with prognosis across WHO grade II-IV gliomas[8,9]. Aim of this study was to explore whether imaging-derived markers such as TTP still add to the profoundly improved prognostic classification realized within the framework of the updated WHO classification[6].

MATERIALS AND METHODS

Patient evaluation

Patients with a supratentorial WHO grade II-IV glioma (WHO 2007) diagnosed between 2004 and 2014 who had undergone ^{18}F-FET-PET with static and dynamic analysis prior to histopathological diagnosis were retrospectively identified. The study was approved by the institutional review board (approval number: 604-16) and all subjects signed a written informed consent as part of the clinical routine. PFS was measured from the date of surgical procedure to the first event of clinical deterioration, i.e. new neurological symptoms, worsening as indicated by Karnofsky

perfomance score (KPS), an increase in steroid medication, or tumor growth on conventional MRI according to modified RANO criteria[10]. OS was correspondingly calculated from date of surgery to date of death. Date of last follow-up was December 2016.

^{18}F-FET-PET and MR imaging

Dynamic ^{18}F-FET-PET scans were acquired with an ECAT EXACT HR+ scanner (Siemens Healthcare, Erlangen, Germany) according to standard protocols after slow intravenous bolus injection of 180 MBq ^{18}F-FET[8]. Dynamic emission recording in 3D-mode consisted of 16 frames (7x10 s, 3x30 s, 1x2 min, 3x5 min, and 2x10 min) and was conducted until 40 minutes post injection. For further evaluation, images were transferred to a HERMES work station (Hermes Medical Solutions, Stockholm, Sweden).

In the semi-quantitative analysis, the maximal tumoral ^{18}F-FET uptake corrected for mean background activity in the contralateral hemisphere (maximal tumor-to-brain-ratio, TBR_{max}) was evaluated. Tumors were termed ^{18}F-FET-"positive" when TBR_{max} was above 1.6[11,12].

For the dynamic analysis, which was performed using HERMES PET Display Dynamic, 90% iso-contour regions of interest were semi-automatically drawn on each individual slice throughout the tumor in the 10-30 minutes summation images and afterwards applied to the dynamic PET data in order to extract the TAC. For each extracted TAC within the tumor, the peak of the curve was identified and the time of peak uptake was noted and set as TTP. This procedure was repeated for all slices throughout the tumor. As explained in detail previously[8], the shortest TTP being present in at least two adjacent slices was defined as minimal TTP (TTP_{min}).

MR imaging was performed prior to tissue sampling according to standard protocols and included acquisition of axial T2-weighted sequences as well as 3D T1-weighted sequences before and after administration of intravenous contrast agent (0.1 mmol/kg gadobenatedimeglumine, MultiHance, BraccoImaging, Milan, Italy). Progression was diagnosed whenever (1) any new contrast enhancement was noted in a previously non-enhancing tumor or (2) T_2 diameter of the tumor enlarged above 25%. Contrast enhancement (CE, yes/no), as well as tumor size and volume (both CE and T_2-based size/volume) were assessed on initial MRI images prior to surgical procedure using Brainlab software (iplan, BrainLab, Heimstetten, Germany).

Surgical procedure

Biopsy or resection was performed according to interdisciplinary tumor board recommendations and patient's preference. The stereotactic biopsy procedure at our institution has been described previously: briefly, multiple tumor tissue specimens were obtained from the area of the highest ^{18}F-FET uptake[13,14]. The microsurgical resection procedure involved MRI- and ^{18}F-FET PET-based neuronavigation (BrainLab, Heimstetten, Germany).

Histopathology and molecular genetic markers

Histological diagnosis was performed by experienced neuropathologists (T.F., A.G.) according to the WHO classification version 2007 blinded for PET and MRI findings[15]. Determination of *MGMT* promoter methylation was performed using methylation-specific PCR[16]. 1p/19q co-deletion was analyzed according to standard protocols[17] with the following microsatellite markers: D1S548, D1S1184, D1S1608,

D1S1592, D1S1161, D19S601, D19S559, D1S433, D19S718, and D19S431. Determination of *IDH1* mutation was performed using pyrosequencing of a 88 bp long fragment of the *IDH1* gene including the mutation hot spot at codon 132, while for *IDH2* mutations, pyrosequencing of a 83 bp long fragment of the *IDH2* gene including the mutation hot spot at codon 172 was performed.

Patients were categorized as either glioblastoma (GBM) or glioma WHO II and III, the latter two groups differentiated by mutational status *IDH* 1/2 and co-deletion 1p/19q.

Statistical analysis

SPSS for Windows (SPSS, Version 21.0, Chicago, IL) was used for statistical calculations. PFS and OS were analyzed with the Kaplan-Meier method; when median PFS or OS times were not reached, mean values were given and used for analysis. The distribution of patient- and tumor-related variables was analyzed by chi-squared statistics (for categorical variables) and Mann-Whitney-U test (for continuously scaled variables). The median was used as threshold for dichotomization of parameters. For univariate prognostic analyses, all parameters were evaluated using Cox regression. Covariates significant in one-variable models were then evaluated in multivariate analyses using a stepwise backwards exclusion model. In case of an inter-correlation of most relevant covariates, alternative models were tested and compared by computing the maximized likelihoods. A two-tailed p-value <0.05 was considered significant.

RESULTS

Patient characteristics

Three-hundred patients (median age 47.6 years (range 8.1-84.0), 166 males) with primary diagnosis of a glioma and ^{18}F-FET PET scan at diagnosis were evaluated. Clinical data of all patients are listed in Table 1. Median follow-up time was 67.5 months for the survivors; during this time period, 178 patients experienced tumor progression and 144 patients died. Median PFS was 23.6 months (95% CI 19.9-27.0) and median OS was 59.3 months (95% CI 29.9-88.6). An *IDH1/2* mutation was found in 142 patients, a 1p/19q co-deletion was present in 60 patients. In 22 patients, information on the molecular tumor profile was not available. Outcome by histological grade versus diagnosis of molecular markers is summarized in Suppl. Table 1. Kaplan-Meier curves for OS by age, gender, WHO grade, *IDH1/2* mutation, and 1p/19q status are summarized in Suppl. Fig 1~~. For PFS and OS by histology and WHO grade in Suppl. Fig. 2.~~ Detailed data on each patient is given in Suppl. Tabl. 2.

^{18}F-FET-PET and MRI correlates of outcome

Initial median tumor size as assessed by T_2-MRI was 49 ml; there was no significant difference in tumor size within the four tumor groups (p=0.33). Contrast enhancement was observed in 172/300 (57%) of all tumors; all glioblastoma (GBM) patients presented with CE, while presence of CE was equally distributed among the three remaining molecular groups (p =0.25). In ^{18}FET-PET, 255 of 300 (85%) tumors had an elevated ^{18}F-FET uptake and thus were classified as ^{18}F-FET-positive. The rates of ^{18}F-FET-negative lesions per molecular subgroup were: 3/58 (5%) for *IDH1/2* mut/1p/19q co-del tumors, 28/79 (35%) for *IDH1/2* mut/1p/19q non co-del tumors, 9/76 (11.8%) for *IDH1/2* wildtype tumors, and 0/73 in GBM (Table 2). In the entire group, a negative ^{18}F-FET-PET was associated with prognosis: PFS and OS

times in patients with ^{18}F-FET-negative tumors were much longer compared to patients with ^{18}F-FET-positive tumors (p=0.01 and 0.001).

Dynamic analysis of tracer uptake could only be performed in ^{18}F-FET-positive tumors. TTP_{min} analysis was therefore not available in the 45 ^{18}F-FET-negative cases and in further 5 patients for technical reasons. Thus, the following calculations refer to a study population of 255 patients. Correlation of continuously scaled TBR_{max} and TTP_{min} with PFS or OS revealed shorter (p<0.001) PFS and OS in patients with higher TBR_{max} and shorter TTP_{min} values. Longer TTP_{min} was highly correlated with the presence of *IDH1/2* mutation (p<0.001). In order to identify different prognostic subgroups, median values for TBR_{max} and TTP_{min} were calculated within the entire ^{18}FET-positive patient population as well as in subgroups defined by molecular markers. PFS and OS were compared between patient groups split by this median.

Median TBR_{max} was 2.6 for all patients. Median PFS and OS times were shorter in patients with TBR_{max} values >2.6 (15.4 (95% CI 12.4 -18.4) / 34.2 (CI 95% 23.0-45.5) months) than in patients with a TBR_{max} $\leq$2.6 (PFS of 50.1 (95% CI 30.0-70.2) months, p<0.001). Mean OS was 89.4 (95% CI 79.9-98.9) months in patients with a TBR_{max} $\leq$2.6 (Table 2).

Median TTP_{min} was 17.5 minutes for all patients; patients with a TTP_{min} >17.5 minutes had longer PFS and OS than patients with a TTP_{min} ≤17.5 minutes: median PFS not reached vs. 14.2 months (95% CI 11.1-17.3)/(log rank p <0.001) and median OS not reached vs. 26.2 months (95% CI 21.6-30.8)/(log rank p <0.001).

Next, we assessed the correlation with outcome of ^{18}F-FET-PET parameters within the molecular subgroups defined by *IDH1/2* mutation and 1p/19q co-deletion status and GBM histology according WHO 2016 classification[6].

***IDH1/2* mut/ 1p /19q co-del group**

In this subgroup, neither presence nor absence of ^{18}F-FET uptake, nor TBR_{max} nor TTP_{min} discriminated between outcome of patients. Median TBR_{max} was 2.7 in this subgroup and median TTP_{min} was 25 minutes. There was no significant OS difference between tumors with a TTP_{min} >25 and those with a TTP_{min} of ≤25 minutes: log-rank p=0.54 for PFS and p=0.92 for OS and (Table 2, Fig. 1 A/B).

IDH 1/2 mut*/ 1p/19q *non co-del tumors

In this subgroup, ^{18}F-FET-uptake per se (positive versus negative) did not correlate with outcome, while the magnitude of ^{18}F-FET uptake did: patients with TBR_{max} values ≤1.7 (in-group median) had a significantly longer PFS time and slightly longer OS time compared to those with TBR_{max} values >1.7 (Table 2). Furthermore, outcome analysis according to the in-group median TTP_{min} of 25 minutes revealed patients with a TTP_{min} >25 minutes to have a significantly better outcome for both PFS and OS (Table 2, Fig.1 C/D). A comparison of clinical parameters between these 2 patient groups with different outcome revealed no distinguishing feature apart from a different distribution of TTP_{min} between the WHO grades (Suppl. Table 3). Interestingly, patients with a TTP_{min} >25 minutes had a better outcome than patients with a TTP_{min} ≤25 minutes irrespective of WHO grade II or III (Fig. 3 B). Median TBR_{max} did not provide a comparable separation (Fig. 3 C). An example of a patient with WHO grade III tumor/ TTP_{min} >25 and favorable outcome as opposed to poor outcome in a patient with a WHO grade II tumor and TTP_{min} ≤25 minutes is illustrated in Suppl. Fig. 2.

***IDH1/2* wt group (WHO grades II and III)**

Neither ^{18}F-FET uptake (positive versus negative) nor median TBR_{max} of 2.5 were associated with outcome (Table 2). Furthermore, outcome analysis according to median TTP_{min} of 12.5 minutes in *IDH1/2* wt tumors showed no difference in PFS or OS times between patients with a TTP_{min} ≤12.5 compared to those with a TTP_{min}>12.5 minutes (p=0.51/p =0.14; Table 2/Fig. 2 A/B).

GBM group

All tumors within this subgroup were ^{18}FET-positive. Neither median TBR_{max} of 3.6 nor the median TTP_{min} of 12.5 minutes was associated with outcome. However, while not reaching statistical significance, with 23.1 months compared to 12.8 months, patients with a TTP_{min} >12.5 minutes had a considerably longer OS time (p = 0.29; Fig. 2 D).

Univariate and multivariate survival analysis (all patients)

Univariate analysis revealed lower age, higher KPS, delay of cytotoxic therapy, lower WHO grade and presence of *IDH1/2* mutation to be highly associated with both PFS and OS in the entire group (Table 3). Absence of CE on initial MRI, absence of ^{18}F-FET uptake, lower TBR_{max} and a TTP_{min} >17.5 min were also associated with longer PFS and OS.

Multivariate analysis was conducted using all parameters with a p-value <0.05 in the univariate analysis. Lower WHO grade and presence of *IDH1/2* mutation were associated with prolonged PFS. In addition to WHO grade and *IDH1/2* mutation, TTP_{min} >17.5 was an independent prognostic factor for improved survival (see Table 3). Subgroup analysis could not be performed due to low number of events in the two *IDH1/2* mutated groups.

DISCUSSION

Since the molecular markers *IDH1/2* mutation and 1p/19q co-deletion have been identified to be strongly associated with prognosis[1-5,18,19 20], the 2016 revision of the WHO Classification of Tumors of the Central Nervous System has implemented a classification scheme for gliomas based on these molecular markers[6]. This might affect therapeutic approaches in the future by allowing stronger emphasis on individual, tumor-tailored therapies based on molecular profiling.

Accordingly, amino-acid PET has been shown to provide valuable information regarding differential diagnosis of cerebral lesions as well as prognosis among gliomas[8,9,12,21,22]. Dynamic analysis of ^{18}F-FET uptake using TTP_{min} analysis discriminates patients with poor or favorable prognosis at the time of diagnosis in gliomas across WHO 2007 grades II to IV[8,9]. In light of the revision of the WHO classification, we sought to reassess the information derived from dynamic analysis of ^{18}F-FET-PET within the framework of a glioma classification in adults largely based on *IDH1/2* mutation and 1p/19q co-deletion.

As a principal observation, longer TTP_{min} correlates with longer OS independently of grading and presence of *IDH1/2* mutation in our entire study population. Notably, in *IDH1/2* mut/1p/19q non co-del gliomas TTP_{min} provides an additional prognostic marker, emphasizing the value of PET in these tumors.

The biological mechanism of tracer kinetics leading to short or long TTP_{min} is not fully understood yet[23]. ^{18}F-FET uptake depends on a bidirectional L-type amino acid transporter (LAT 1/2) expressed in the cell membrane and vasculature of gliomas. Its expression level was found to correlate with the degree of malignancy according to the WHO 2007 classification: pooled grade III and IV gliomas had much higher LAT1

expression than WHO grade II gliomas[24]. Moreover, overexpression of LAT1 in glioma cells with low endogenous LAT1 expression enhanced tumor growth in nude mice[24]. After intracellular uptake, ^{18}F-FET is not incorporated into proteins or trapped within the tumor cell, but washed out after a certain period of time[23]. The faster the ^{18}F-FET uptake, the faster it is washed out of the tumor cell. A higher tracer turnover might be influenced by either a higher LAT1/2 expression or higher tracer availability due to increased tumor vascularity/perfusion. High vascularity and an elevated ratio of LAT transporters have been reported for gliomas WHO grade III and IV gliomas, both might contribute to this observation. Both patients with *IDH1/2* wildtype WHO II and III tumors as well as GBM patients have the shortest median TTP_{min} of 12.5 minutes. In the GBM subgroup, patients with a TTP_{min} >12.5 minutes have a considerably longer survival time of 23.1 months compared to 12.8 months in patients with a TTP_{min} ≤12.5. In the other group also known to display higher perfusion, namely the *IDH1/2* mut/1p/19 q co-del tumors, we did not detect TTP_{min} as a prognostic factor, which might be due to a limited number of events. One could speculate whether high perfusion might interfere with effects of TTP_{min} in these two highly vascularized tumor groups. Interestingly, different perfusion properties were shown to be associated with both outcome and presence of *IDH1/2* mutation in astrocytic tumors by Kickingereder et al[25]. These authors found an over-activation of pro-angiogenic pathways in *IDH1/2* wt tumors, well explaining the observed difference in rCBV in the different molecular subgroups and demonstrating the potential additional value of imaging biomarkers. As TTP_{min} was associated with survival in tumors being *IDH1/2* mutated without 1p/19q co-deletion, both TTP_{min} and perfusion might be surrogates of a distinct biological tumor property. Further studies

combining dynamic PET and perfusion-based MRI may help elucidating the interaction between perfusion, vascularity and TTP_{min}.

In contrast to TTP_{min}, TBR_{max} was associated with prognosis in the two *IDH1/2* mutant groups: OS was longer, albeit not significant, in *IDH1/2* mut/co-del 1p/19q patients with $TBR_{max} \leq 2.7$ (p=0.07) and PFS was significantly longer in the non-co-del tumors with $TBR_{max} \leq 1.7$ (p=0.01. Hence, the magnitude of ^{18}F-FET uptake might be associated with histological features such as cell density, mitotic index or vascularization and thus most likely reflects WHO grade [26].

The factor "^{18}F-FET-negative" was associated with favorable outcome in the entire group, however, this could be attributed to the high inter-correlation with the molecular subtype: ^{18}F-FET-negative tumors were most often found among the *IDH1/2* mut/1p/19q non co-del (astrocytic) tumor type, while 95% of *IDH1/2* wt tumors were ^{18}F-FET-positive.

Although counterintuitive, the factor "^{18}F-FET-negative" lost its significance within the group of *IDH1/2* mut/1p/19q no co-del tumors, and could not be evaluated in the remaining two groups due to small number of ^{18}F-FET-negative cases. So far, the underlying mechanisms leading to complete lack of ^{18}F-FET-uptake are not understood yet; one explanation might be the lack or an inactivity of LAT transporters in a proportion of *IDH1/2* mut/1p/19q no co-del tumors (approximately one third of our astrocytic tumor population) and remains to be addressed in further studies.

Limitations of the study arise from the retrospective study design and the heterogeneous surgical and post-surgical management strategies.

Furthermore, TBR_{max} values as well as determination of TTP_{min} are dependent on scanner resolution and data processing, with the consequence that absolute values

may not be comparable between different centers[22,27]. A standardization of data processing and evaluation will help to improve comparability.

Altogether, dynamic analysis of ^{18}F-FET tracer uptake using TTP_{min} discriminates patients with favorable and poor prognosis within the molecular defined subgroup of *IDH1/2* mut/1p/19q non co-del tumors, most of which are now classified as astrocytomas. Thus, this might be an imaging biomarker providing additional prognostic information to stratify astrocytoma patients into low risk and high-risk groups.

REFERENCES

1. Weller M, Pfister SM, Wick W, Hegi ME, Reifenberger G, Stupp R. Molecular neuro-oncology in clinical practice: a new horizon. *Lancet Oncol.* Aug 2013;14(9):e370-379.

2. Weller M, Weber RG, Willscher E, et al. Molecular classification of diffuse cerebral WHO grade II/III gliomas using genome- and transcriptome-wide profiling improves stratification of prognostically distinct patient groups. *Acta neuropathologica.* May 2015;129(5):679-693.

3. Cancer Genome Atlas Research N, Brat DJ, Verhaak RG, et al. Comprehensive, Integrative Genomic Analysis of Diffuse Lower-Grade Gliomas. *The New England journal of medicine.* Jun 25 2015;372(26):2481-2498.

4. Eckel-Passow JE, Lachance DH, Molinaro AM, et al. Glioma Groups Based on 1p/19q, IDH, and TERT Promoter Mutations in Tumors. *The New England journal of medicine.* Jun 25 2015;372(26):2499-2508.

5. Suzuki H, Aoki K, Chiba K, et al. Mutational landscape and clonal architecture in grade II and III gliomas. *Nature genetics.* May 2015;47(5):458-468.

6. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathologica.* Jun 2016;131(6):803-820.

7. Suchorska B, Jansen NL, Linn J, et al. Biological tumor volume in 18FET-PET before radiochemotherapy correlates with survival in GBM. *Neurology.* Feb 17 2015;84(7):710-719.

8. Jansen NL, Suchorska B, Wenter V, et al. Prognostic significance of dynamic 18F-FET PET in newly diagnosed astrocytic high-grade glioma. *Journal of nuclear medicine.* Jan 2015;56(1):9-15.

9. Jansen NL, Suchorska B, Wenter V, et al. Dynamic 18F-FET PET in newly diagnosed astrocytic low-grade glioma identifies high-risk patients. *Journal of Nuclear Medicine.* Feb 2014;55(2):198-203.

10. Wen PY, Macdonald DR, Reardon DA, et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. *Journal of clinical oncology.* Apr 10 2010;28(11):1963-1972.

11. Unterrainer M, Schweisthal F, Suchorska B, et al. Serial 18F-FET PET Imaging of Primarily 18F-FET-Negative Glioma: Does It Make Sense? *Journal of Nuclear Medicine.* Aug 2016;57(8):1177-1182.

12. Floeth FW, Pauleit D, Sabel M, et al. Prognostic value of O-(2-18F-fluoroethyl)-L-tyrosine PET and MRI in low-grade glioma. *Journal of Nuclear Medicine.* Apr 2007;48(4):519-527.

13. Kunz M, Thon N, Eigenbrod S, et al. Hot spots in dynamic (18)FET-PET delineate malignant tumor parts within suspected WHO grade II gliomas. *Neuro-oncology.* Mar 2011;13(3):307-316.

14. Eigenbrod S, Trabold R, Brucker D, et al. Molecular stereotactic biopsy technique improves diagnostic accuracy and enables personalized treatment strategies in glioma patients. *Acta neurochirurgica.* Aug 2014;156(8):1427-1440.

15. Louis DN, Ohgaki H, Wiestler OD, et al. The 2007 WHO classification of tumours of the central nervous system. *Acta neuropathologica.* Aug 2007;114(2):97-109.

16. Grasbon-Frodl EM, Kreth FW, Ruiter M, et al. Intratumoral homogeneity of MGMT promoter hypermethylation as demonstrated in serial stereotactic specimens from anaplastic astrocytomas and glioblastomas. *International journal of cancer.* Dec 01 2007;121(11):2458-2464.

17. Thon N, Eigenbrod S, Grasbon-Frodl EM, et al. Novel molecular stereotactic biopsy procedures reveal intratumoral homogeneity of loss of heterozygosity of 1p/19q and TP53 mutations in World Health Organization grade II gliomas. *Journal of Neuropathology Experimental Neurology.* Nov 2009;68(11):1219-1228.

18. Van Den Bent MJ, Bromberg JE, Buckner J. Low-grade and anaplastic oligodendroglioma. *Handbook of clinical neurology.* 2016;134:361-380.

19. van den Bent MJ, Brandes AA, Taphoorn MJ, et al. Adjuvant procarbazine, lomustine, and vincristine chemotherapy in newly diagnosed anaplastic oligodendroglioma: long-term follow-up of EORTC brain tumor group study 26951. *Journal of clinical oncology.* Jan 20 2013;31(3):344-350.

20. Cairncross G, Wang M, Shaw E, et al. Phase III trial of chemoradiotherapy for anaplastic oligodendroglioma: long-term results of RTOG 9402. *Journal of clinical oncology.* Jan 20 2013;31(3):337-343.

21. Jansen NL, Graute V, Armbruster L, et al. MRI-suspected low-grade glioma: is there a need to perform dynamic FET PET? *European Journal of Nuclear Medicine and Molecular Imaging.* Jun 2012;39(6):1021-1029.

22. Albert NL, Weller M, Suchorska B, et al. Response Assessment in Neuro-Oncology working group and European Association for Neuro-Oncology recommendations for the clinical use of PET imaging in gliomas. *Neuro-oncology.* Sep 2016;18(9):1199-1208.

23. Langen KJ, Hamacher K, Weckesser M, et al. O-(2-[18F]fluoroethyl)-L-tyrosine: uptake mechanisms and clinical applications. *Nuclear medicine and biology.* Apr 2006;33(3):287-294.

24. Kobayashi K, Ohnishi A, Promsuk J, et al. Enhanced tumor growth elicited by L-type amino acid transporter 1 in human malignant glioma cells. *Neurosurgery.* Feb 2008;62(2):493-503; discussion 503-494.

25. Kickingereder P, Sahm F, Radbruch A, et al. IDH mutation status is associated with a distinct hypoxia/angiogenesis transcriptome signature which is non-invasively predictable with rCBV imaging in human glioma. *Scientific reports.* Nov 05 2015;5:16238.

26. Rapp M, Heinzel A, Galldiks N, et al. Diagnostic performance of 18F-FET PET in newly diagnosed cerebral lesions suggestive of glioma.*Journal of Nuclear Medicine.* Feb 2013;54(2):229-235.

27. Langen KJ, Galldiks N, Hattingen E, Shah NJ. Advances in neuro-oncology imaging. *Nature Reviews Neurology.* May 2017;13(5):279-289.

Figures:

Figure 1. Correlation of Progression Free and Overall Survival Times with the in-group TTP_{min} median within the *IDH 1/2* mut/1p/19q co-del (**A**/**B**) and the *IDH 1/2* mut/1p/19q non-codel (**C**/**D)** group

Figure 2: Correlation of Progression Free and Overall Survival Times with the in-group TTP_{min} median within the *IDH 1/2* wt (**A**/**B**) and the GBM (**C**/**D)** group

Figure 3. Overall Survival times according to WHO 2007 classification (**A**), according to median TBR_{max} values within the WHO grade II and III groups (**B**) and to median TTP_{min} values within the WHO grade II und III groups (**C**) within the *IDH 1/2* mut/1p/19q non-codel group.

Supplemental Figure 1: Overall Survival by age ≤48 years vs. >48 years (**A**); gender (**B**); WHO grade (**C**), *IDH1/2* mutation status (**D**) and IDH1/2 mutation and 1p/19q status and GBM histology (**E**)

Supplemental Figure 2: Example of a patient with an (astrocytic) *IDH1/2* mut/no 1p/19q co-del WHO III tumor with a long time-to-peak time (TTP_{min} >25) minutes and a favorable outcome; following resection and chemotherapy, the patient is still alive after 69 months without further therapy (**A**) In contrast, an example of a patient with an (astrocytic) *IDH1/2* mut/no 1p/19q co-del WHO II tumor and a short time-to-peak (TTP_{min}≤ 25 min) who died after 73 months following biopsy, chemotherapy, as well as multiple salvage-therapies for progressive disease (**B**).

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Identification of time-to-peak on dynamic ^{18}F-FET-PET as a prognostic marker specifically in *IDH1/2* mutant diffuse astrocytoma

Running title: TTP as prognostic marker in IDH mutant astrocytoma

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Abstract

Background: Stratification of glioma according to isocitrate dehydrogenase 1/2 (*IDH1/2)* mutation and 1p/19q co-deletion status has gained major importance in the new WHO classification. Parameters derived from ^{18}F-FET-PET uptake dynamics such as minimal time-to-peak (TTP_{min}) allow discrimination between different prognostic glioma subgroups, too. The present study aimed at exploring whether TTP_{min} analysis provides prognostic information beyond the WHO classification.

Methods: Three-hundred patients with newly diagnosed WHO 2007 grade II-IV gliomas with ^{18}F-FET-PET imaging at diagnosis were grouped into 4 subgroups (*IDH1/2* mut/1p/19q co-del; *IDH1/2* mut/1p/19q non co-del, *IDH1/2* wildtype WHO grade II and III tumors, and glioblastoma). Clinical and imaging factors such as age, Karnofsky performance score, treatment, TTP_{min} and maximal tumor-to-brain ratio (TBR_{max}) were analyzed with regard to progression-free and overall survival (PFS and OS) via univariate and multivariate regression analysis.

Results: PFS and OS were longest in the *IDH1/2* mut/1p/19q co-del subgroup followed by *IDH1/2* mut/1p/19q non co-del, *IDH1/2* wt patients and GBM ($p<0.001$). Further, outcome stratified by TTP_{min} with a cutoff of 17.5 minutes revealed significantly longer PFS and OS in patients with TTP_{min} >17.5 minutes ($p<0.001$ for PFS and OS). Lower TBR_{max} values or the absence of ^{18}F-FET-uptake were also associated with favorable outcome in the entire group. In the subgroup analyses, longer median TTP_{min} was associated with improved outcome specifically in the *IDH1/2* mut/1p/19q non co-del group.

Conclusion: ^{18}F-FET-PET-derived dynamic analysis defines prognostically distinct sub-groups of *IDH1/2* mutant/ 1p/19q-non-co-deleted gliomas which cannot be distinguished as yet by molecular marker analysis**.**

Importance of the study:

In light of the revised WHO 2016 classification, management of gliomas will increasingly depend on their molecular genetic profile. Data from more refined imaging techniques, such as the dynamic ^{18}F-FET-PET uptake analysis discussed in this manuscript, indicate that imaging biomarkers might provide additional information relevant for prognosis. The current manuscript explores the value of dynamic ^{18}F-FET-PET in 300 patients with a newly diagnosed WHO grade II-IV glioma; our data show dynamic uptake analysis reflected by time-to-peak (TTP) to provide further prognostic information within molecular subgroups according to *IDH1/2* mutation and co-deletion 1p/19q independently of WHO grading.

INTRODUCTION

Tailoring treatment options in glioma patients according to an individual risk profile is gaining increasing importance in the field of neuro-oncology[1]. Recent analyses of large cohort studies have uncovered a dominant association of molecular markers with clinical outcome in glioma patients[2-5]: As a consequence, the 2016 revision of

the WHO classification of tumors of the central nervous system accounts for molecular markers for sub-classification of gliomas[6]: *IDH1/2* mutant tumors have a different biology and better outcome than *IDH1/2* wildtype tumors, and *IDH1/2* mutant tumors are further subdivided into 1p/19q-co-deleted – associated with oligodendroglial morphology and better outcome – and non-co-deleted tumors – associated with astrocytic morphology and intermediate outcome.

Recent advances in molecular imaging via *O*-(2-^{18}F-fluorethyl)-L-tyrosine positron emission tomography (^{18}F-FET-PET) have led to the establishment of several non-invasive, imaging-derived prognostic factors such as biological tumor volume (BTV) and dynamic tracer uptake represented by time activity curves (TAC) and time-to-peak (TTP) evaluation[7-9]. In particular the dynamic evaluation of uptake including TTP analysis is highly associated with prognosis across WHO grade II-IV gliomas[8,9]. Aim of this study was to explore whether imaging-derived markers such as TTP still add to the profoundly improved prognostic classification realized within the framework of the updated WHO classification[6].

MATERIALS AND METHODS

Patient evaluation

Patients with a supratentorial WHO grade II-IV glioma (WHO 2007) diagnosed between 2004 and 2014 who had undergone ^{18}F-FET-PET with static and dynamic analysis prior to histopathological diagnosis were retrospectively identified. The study was approved by the institutional review board (approval number: 604-16) and all subjects signed a written informed consent as part of the clinical routine. PFS was measured from the date of surgical procedure to the first event of clinical deterioration, i.e. new neurological symptoms, worsening as indicated by Karnofsky

perfomance score (KPS), an increase in steroid medication, or tumor growth on conventional MRI according to modified RANO criteria[10]. OS was correspondingly calculated from date of surgery to date of death. Date of last follow-up was December 2016.

^{18}F-FET-PET and MR imaging

Dynamic ^{18}F-FET-PET scans were acquired with an ECAT EXACT HR+ scanner (Siemens Healthcare, Erlangen, Germany) according to standard protocols after slow intravenous bolus injection of 180 MBq ^{18}F-FET[8]. Dynamic emission recording in 3D-mode consisted of 16 frames (7x10 s, 3x30 s, 1x2 min, 3x5 min, and 2x10 min) and was conducted until 40 minutes post injection. For further evaluation, images were transferred to a HERMES work station (Hermes Medical Solutions, Stockholm, Sweden).

In the semi-quantitative analysis, the maximal tumoral ^{18}F-FET uptake corrected for mean background activity in the contralateral hemisphere (maximal tumor-to-brain-ratio, TBR_{max}) was evaluated. Tumors were termed ^{18}F-FET-"positive" when TBR_{max} was above 1.6[11,12].

For the dynamic analysis, which was performed using HERMES PET Display Dynamic, 90% iso-contour regions of interest were semi-automatically drawn on each individual slice throughout the tumor in the 10-30 minutes summation images and afterwards applied to the dynamic PET data in order to extract the TAC. For each extracted TAC within the tumor, the peak of the curve was identified and the time of peak uptake was noted and set as TTP. This procedure was repeated for all slices throughout the tumor. As explained in detail previously[8], the shortest TTP being present in at least two adjacent slices was defined as minimal TTP (TTP_{min}).

MR imaging was performed prior to tissue sampling according to standard protocols and included acquisition of axial T2-weighted sequences as well as 3D T1-weighted sequences before and after administration of intravenous contrast agent (0.1 mmol/kg gadobenatedimeglumine, MultiHance, BraccoImaging, Milan, Italy). Progression was diagnosed whenever (1) any new contrast enhancement was noted in a previously non-enhancing tumor or (2) T_2 diameter of the tumor enlarged above 25%. Contrast enhancement (CE, yes/no), as well as tumor size and volume (both CE and T_2-based size/volume) were assessed on initial MRI images prior to surgical procedure using Brainlab software (iplan, BrainLab, Heimstetten, Germany).

Surgical procedure

Biopsy or resection was performed according to interdisciplinary tumor board recommendations and patient's preference. The stereotactic biopsy procedure at our institution has been described previously: briefly, multiple tumor tissue specimens were obtained from the area of the highest ^{18}F-FET uptake[13,14]. The microsurgical resection procedure involved MRI- and ^{18}F-FET PET-based neuronavigation (BrainLab, Heimstetten, Germany).

Histopathology and molecular genetic markers

Histological diagnosis was performed by experienced neuropathologists (T.F., A.G.) according to the WHO classification version 2007 blinded for PET and MRI findings[15]. Determination of *MGMT* promoter methylation was performed using methylation-specific PCR[16]. 1p/19q co-deletion was analyzed according to standard protocols[17] with the following microsatellite markers: D1S548, D1S1184, D1S1608,

D1S1592, D1S1161, D19S601, D19S559, D1S433, D19S718, and D19S431. Determination of *IDH1* mutation was performed using pyrosequencing of a 88 bp long fragment of the *IDH1* gene including the mutation hot spot at codon 132, while for *IDH2* mutations, pyrosequencing of a 83 bp long fragment of the *IDH2* gene including the mutation hot spot at codon 172 was performed.

Patients were categorized as either glioblastoma (GBM) or glioma WHO II and III, the latter two groups differentiated by mutational status *IDH* 1/2 and co-deletion 1p/19q.

Statistical analysis

SPSS for Windows (SPSS, Version 21.0, Chicago, IL) was used for statistical calculations. PFS and OS were analyzed with the Kaplan-Meier method; when median PFS or OS times were not reached, mean values were given and used for analysis. The distribution of patient- and tumor-related variables was analyzed by chi-squared statistics (for categorical variables) and Mann-Whitney-U test (for continuously scaled variables). The median was used as threshold for dichotomization of parameters. For univariate prognostic analyses, all parameters were evaluated using Cox regression. Covariates significant in one-variable models were then evaluated in multivariate analyses using a stepwise backwards exclusion model. In case of an inter-correlation of most relevant covariates, alternative models were tested and compared by computing the maximized likelihoods. A two-tailed p-value <0.05 was considered significant.

RESULTS

Patient characteristics

Three-hundred patients (median age 47.6 years (range 8.1-84.0), 166 males) with primary diagnosis of a glioma and ^{18}F-FET PET scan at diagnosis were evaluated. Clinical data of all patients are listed in Table 1. Median follow-up time was 67.5 months for the survivors; during this time period, 178 patients experienced tumor progression and 144 patients died. Median PFS was 23.6 months (95% CI 19.9-27.0) and median OS was 59.3 months (95% CI 29.9-88.6). An *IDH1/2* mutation was found in 142 patients, a 1p/19q co-deletion was present in 60 patients. In 22 patients, information on the molecular tumor profile was not available. Outcome by histological grade versus diagnosis of molecular markers is summarized in Suppl. Table 1. Kaplan-Meier curves for OS by age, gender, WHO grade, *IDH1/2* mutation, and 1p/19q status are summarized in Suppl. Fig 1. Detailed data on each patient is given in Suppl. Tabl. 2.

^{18}F-FET-PET and MRI correlates of outcome

Initial median tumor size as assessed by T_2-MRI was 49 ml; there was no significant difference in tumor size within the four tumor groups (p=0.33). Contrast enhancement was observed in 172/300 (57%) of all tumors; all glioblastoma (GBM) patients presented with CE, while presence of CE was equally distributed among the three remaining molecular groups (p =0.25). In ^{18}FET-PET, 255 of 300 (85%) tumors had an elevated ^{18}F-FET uptake and thus were classified as ^{18}F-FET-positive. The rates of ^{18}F-FET-negative lesions per molecular subgroup were: 3/58 (5%) for *IDH1/2* mut/1p/19q co-del tumors, 28/79 (35%) for *IDH1/2* mut/1p/19q non co-del tumors, 9/76 (11.8%) for *IDH1/2* wildtype tumors, and 0/73 in GBM (Table 2). In the entire group, a negative ^{18}F-FET-PET was associated with prognosis: PFS and OS

times in patients with ^{18}F-FET-negative tumors were much longer compared to patients with ^{18}F-FET-positive tumors (p=0.01 and 0.001).

Dynamic analysis of tracer uptake could only be performed in ^{18}F-FET-positive tumors. TTP_{min} analysis was therefore not available in the 45 ^{18}F-FET-negative cases and in further 5 patients for technical reasons. Thus, the following calculations refer to a study population of 255 patients. Correlation of continuously scaled TBR_{max} and TTP_{min} with PFS or OS revealed shorter (p<0.001) PFS and OS in patients with higher TBR_{max} and shorter TTP_{min} values. Longer TTP_{min} was highly correlated with the presence of *IDH1/2* mutation (p<0.001). In order to identify different prognostic subgroups, median values for TBR_{max} and TTP_{min} were calculated within the entire ^{18}FET-positive patient population as well as in subgroups defined by molecular markers. PFS and OS were compared between patient groups split by this median.

Median TBR_{max} was 2.6 for all patients. Median PFS and OS times were shorter in patients with TBR_{max} values >2.6 (15.4 (95% CI 12.4 -18.4) / 34.2 (CI 95% 23.0-45.5) months) than in patients with a $TBR_{max} \leq 2.6$ (PFS of 50.1 (95% CI 30.0-70.2) months, p<0.001). Mean OS was 89.4 (95% CI 79.9-98.9) months in patients with a $TBR_{max} \leq 2.6$ (Table 2).

Median TTP_{min} was 17.5 minutes for all patients; patients with a TTP_{min} >17.5 minutes had longer PFS and OS than patients with a TTP_{min} ≤17.5 minutes: median PFS not reached vs. 14.2 months (95% CI 11.1-17.3)/(log rank p <0.001) and median OS not reached vs. 26.2 months (95% CI 21.6-30.8)/(log rank p <0.001).

Next, we assessed the correlation with outcome of ^{18}F-FET-PET parameters within the molecular subgroups defined by *IDH1/2* mutation and 1p/19q co-deletion status and GBM histology according WHO 2016 classification[6].

***IDH1/2* mut/ 1p /19q co-del group**

In this subgroup, neither presence nor absence of ^{18}F-FET uptake, nor TBR_{max} nor TTP_{min} discriminated between outcome of patients. Median TBR_{max} was 2.7 in this subgroup and median TTP_{min} was 25 minutes. There was no significant OS difference between tumors with a TTP_{min} >25 and those with a TTP_{min} of ≤25 minutes: log-rank p=0.54 for PFS and p=0.92 for OS and (Table 2, Fig. 1 A/B).

IDH 1/2 mut/* 1p/19q *non co-del tumors

In this subgroup, ^{18}F-FET-uptake per se (positive versus negative) did not correlate with outcome, while the magnitude of ^{18}F-FET uptake did: patients with TBR_{max} values ≤1.7 (in-group median) had a significantly longer PFS time and slightly longer OS time compared to those with TBR_{max} values >1.7 (Table 2). Furthermore, outcome analysis according to the in-group median TTP_{min} of 25 minutes revealed patients with a TTP_{min} >25 minutes to have a significantly better outcome for both PFS and OS (Table 2, Fig.1 C/D). A comparison of clinical parameters between these 2 patient groups with different outcome revealed no distinguishing feature apart from a different distribution of TTP_{min} between the WHO grades (Suppl. Table 3). Interestingly, patients with a TTP_{min} >25 minutes had a better outcome than patients with a TTP_{min} ≤25 minutes irrespective of WHO grade II or III (Fig. 3 B). Median TBR_{max} did not provide a comparable separation (Fig. 3 C). An example of a patient with WHO grade III tumor/ TTP_{min} >25 and favorable outcome as opposed to poor outcome in a patient with a WHO grade II tumor and TTP_{min} ≤25 minutes is illustrated in Suppl. Fig. 2.

***IDH1/2* wt group (WHO grades II and III)**

Neither ^{18}F-FET uptake (positive versus negative) nor median TBR_{max} of 2.5 were associated with outcome (Table 2). Furthermore, outcome analysis according to median TTP_{min} of 12.5 minutes in *IDH1/2* wt tumors showed no difference in PFS or OS times between patients with a TTP_{min} ≤12.5 compared to those with a TTP_{min}>12.5 minutes (p=0.51/p =0.14; Table 2/Fig. 2 A/B).

GBM group

All tumors within this subgroup were ^{18}FET-positive. Neither median TBR_{max} of 3.6 nor the median TTP_{min} of 12.5 minutes was associated with outcome. However, while not reaching statistical significance, with 23.1 months compared to 12.8 months, patients with a TTP_{min} >12.5 minutes had a considerably longer OS time (p = 0.29; Fig. 2 D).

Univariate and multivariate survival analysis (all patients)

Univariate analysis revealed lower age, higher KPS, delay of cytotoxic therapy, lower WHO grade and presence of *IDH1/2* mutation to be highly associated with both PFS and OS in the entire group (Table 3). Absence of CE on initial MRI, absence of ^{18}F-FET uptake, lower TBR_{max} and a TTP_{min} >17.5 min were also associated with longer PFS and OS.

Multivariate analysis was conducted using all parameters with a p-value <0.05 in the univariate analysis. Lower WHO grade and presence of *IDH1/2* mutation were associated with prolonged PFS. In addition to WHO grade and *IDH1/2* mutation, TTP_{min} >17.5 was an independent prognostic factor for improved survival (see Table 3). Subgroup analysis could not be performed due to low number of events in the two *IDH1/2* mutated groups.

DISCUSSION

Since the molecular markers *IDH1/2* mutation and 1p/19q co-deletion have been identified to be strongly associated with prognosis[1-5,18,19 20], the 2016 revision of the WHO Classification of Tumors of the Central Nervous System has implemented a classification scheme for gliomas based on these molecular markers[6]. This might affect therapeutic approaches in the future by allowing stronger emphasis on individual, tumor-tailored therapies based on molecular profiling.

Accordingly, amino-acid PET has been shown to provide valuable information regarding differential diagnosis of cerebral lesions as well as prognosis among gliomas[8,9,12,21,22]. Dynamic analysis of ^{18}F-FET uptake using TTP_{min} analysis discriminates patients with poor or favorable prognosis at the time of diagnosis in gliomas across WHO 2007 grades II to IV[8,9]. In light of the revision of the WHO classification, we sought to reassess the information derived from dynamic analysis of ^{18}F-FET-PET within the framework of a glioma classification in adults largely based on *IDH1/2* mutation and 1p/19q co-deletion.

As a principal observation, longer TTP_{min} correlates with longer OS independently of grading and presence of *IDH1/2* mutation in our entire study population. Notably, in *IDH1/2* mut/1p/19q non co-del gliomas TTP_{min} provides an additional prognostic marker, emphasizing the value of PET in these tumors.

The biological mechanism of tracer kinetics leading to short or long TTP_{min} is not fully understood yet[23]. ^{18}F-FET uptake depends on a bidirectional L-type amino acid transporter (LAT 1/2) expressed in the cell membrane and vasculature of gliomas. Its expression level was found to correlate with the degree of malignancy according to the WHO 2007 classification: pooled grade III and IV gliomas had much higher LAT1

expression than WHO grade II gliomas[24]. Moreover, overexpression of LAT1 in glioma cells with low endogenous LAT1 expression enhanced tumor growth in nude mice[24]. After intracellular uptake, ^{18}F-FET is not incorporated into proteins or trapped within the tumor cell, but washed out after a certain period of time[23]. The faster the ^{18}F-FET uptake, the faster it is washed out of the tumor cell. A higher tracer turnover might be influenced by either a higher LAT1/2 expression or higher tracer availability due to increased tumor vascularity/perfusion. High vascularity and an elevated ratio of LAT transporters have been reported for gliomas WHO grade III and IV gliomas, both might contribute to this observation. Both patients with *IDH1/2* wildtype WHO II and III tumors as well as GBM patients have the shortest median TTP_{min} of 12.5 minutes. In the GBM subgroup, patients with a TTP_{min} >12.5 minutes have a considerably longer survival time of 23.1 months compared to 12.8 months in patients with a TTP_{min} ≤12.5. In the other group also known to display higher perfusion, namely the *IDH1/2* mut/1p/19 q co-del tumors, we did not detect TTP_{min} as a prognostic factor, which might be due to a limited number of events. One could speculate whether high perfusion might interfere with effects of TTP_{min} in these two highly vascularized tumor groups. Interestingly, different perfusion properties were shown to be associated with both outcome and presence of *IDH1/2* mutation in astrocytic tumors by Kickingereder et al[25]. These authors found an over-activation of pro-angiogenic pathways in *IDH1/2* wt tumors, well explaining the observed difference in rCBV in the different molecular subgroups and demonstrating the potential additional value of imaging biomarkers. As TTP_{min} was associated with survival in tumors being *IDH1/2* mutated without 1p/19q co-deletion, both TTP_{min} and perfusion might be surrogates of a distinct biological tumor property. Further studies

combining dynamic PET and perfusion-based MRI may help elucidating the interaction between perfusion, vascularity and TTP_{min}.

In contrast to TTP_{min}, TBR_{max} was associated with prognosis in the two *IDH1/2* mutant groups: OS was longer, albeit not significant, in *IDH1/2* mut/co-del 1p/19q patients with $TBR_{max} \leq 2.7$ (p=0.07) and PFS was significantly longer in the non-co-del tumors with $TBR_{max} \leq 1.7$ (p=0.01. Hence, the magnitude of ^{18}F-FET uptake might be associated with histological features such as cell density, mitotic index or vascularization and thus most likely reflects WHO grade [26].

The factor "^{18}F-FET-negative" was associated with favorable outcome in the entire group, however, this could be attributed to the high inter-correlation with the molecular subtype: ^{18}F-FET-negative tumors were most often found among the *IDH1/2* mut/1p/19q non co-del (astrocytic) tumor type, while 95% of *IDH1/2* wt tumors were ^{18}F-FET-positive.

Although counterintuitive, the factor "^{18}F-FET-negative" lost its significance within the group of *IDH1/2* mut/1p/19q no co-del tumors, and could not be evaluated in the remaining two groups due to small number of ^{18}F-FET-negative cases. So far, the underlying mechanisms leading to complete lack of ^{18}F-FET-uptake are not understood yet; one explanation might be the lack or an inactivity of LAT transporters in a proportion of *IDH1/2* mut/1p/19q no co-del tumors (approximately one third of our astrocytic tumor population) and remains to be addressed in further studies.

Limitations of the study arise from the retrospective study design and the heterogeneous surgical and post-surgical management strategies.

Furthermore, TBR_{max} values as well as determination of TTP_{min} are dependent on scanner resolution and data processing, with the consequence that absolute values

may not be comparable between different centers[22,27]. A standardization of data processing and evaluation will help to improve comparability.

Altogether, dynamic analysis of ^{18}F-FET tracer uptake using TTP_{min} discriminates patients with favorable and poor prognosis within the molecular defined subgroup of *IDH1/2* mut/1p/19q non co-del tumors, most of which are now classified as astrocytomas. Thus, this might be an imaging biomarker providing additional prognostic information to stratify astrocytoma patients into low risk and high-risk groups.

REFERENCES

1. Weller M, Pfister SM, Wick W, Hegi ME, Reifenberger G, Stupp R. Molecular neuro-oncology in clinical practice: a new horizon. *Lancet Oncol.* Aug 2013;14(9):e370-379.

2. Weller M, Weber RG, Willscher E, et al. Molecular classification of diffuse cerebral WHO grade II/III gliomas using genome- and transcriptome-wide profiling improves stratification of prognostically distinct patient groups. *Acta neuropathologica.* May 2015;129(5):679-693.

3. Cancer Genome Atlas Research N, Brat DJ, Verhaak RG, et al. Comprehensive, Integrative Genomic Analysis of Diffuse Lower-Grade Gliomas. *The New England journal of medicine.* Jun 25 2015;372(26):2481-2498.

4. Eckel-Passow JE, Lachance DH, Molinaro AM, et al. Glioma Groups Based on 1p/19q, IDH, and TERT Promoter Mutations in Tumors. *The New England journal of medicine.* Jun 25 2015;372(26):2499-2508.

5. Suzuki H, Aoki K, Chiba K, et al. Mutational landscape and clonal architecture in grade II and III gliomas. *Nature genetics.* May 2015;47(5):458-468.

6. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathologica.* Jun 2016;131(6):803-820.

7. Suchorska B, Jansen NL, Linn J, et al. Biological tumor volume in 18FET-PET before radiochemotherapy correlates with survival in GBM. *Neurology.* Feb 17 2015;84(7):710-719.

8. Jansen NL, Suchorska B, Wenter V, et al. Prognostic significance of dynamic 18F-FET PET in newly diagnosed astrocytic high-grade glioma. *Journal of nuclear medicine.* Jan 2015;56(1):9-15.

9. Jansen NL, Suchorska B, Wenter V, et al. Dynamic 18F-FET PET in newly diagnosed astrocytic low-grade glioma identifies high-risk patients. *Journal of Nuclear Medicine.* Feb 2014;55(2):198-203.

10. Wen PY, Macdonald DR, Reardon DA, et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. *Journal of clinical oncology.* Apr 10 2010;28(11):1963-1972.

11. Unterrainer M, Schweisthal F, Suchorska B, et al. Serial 18F-FET PET Imaging of Primarily 18F-FET-Negative Glioma: Does It Make Sense? *Journal of Nuclear Medicine.* Aug 2016;57(8):1177-1182.

12. Floeth FW, Pauleit D, Sabel M, et al. Prognostic value of O-(2-18F-fluoroethyl)-L-tyrosine PET and MRI in low-grade glioma. *Journal of Nuclear Medicine.* Apr 2007;48(4):519-527.

13. Kunz M, Thon N, Eigenbrod S, et al. Hot spots in dynamic (18)FET-PET delineate malignant tumor parts within suspected WHO grade II gliomas. *Neuro-oncology.* Mar 2011;13(3):307-316.

14. Eigenbrod S, Trabold R, Brucker D, et al. Molecular stereotactic biopsy technique improves diagnostic accuracy and enables personalized treatment strategies in glioma patients. *Acta neurochirurgica.* Aug 2014;156(8):1427-1440.

15. Louis DN, Ohgaki H, Wiestler OD, et al. The 2007 WHO classification of tumours of the central nervous system. *Acta neuropathologica.* Aug 2007;114(2):97-109.

16. Grasbon-Frodl EM, Kreth FW, Ruiter M, et al. Intratumoral homogeneity of MGMT promoter hypermethylation as demonstrated in serial stereotactic specimens from anaplastic astrocytomas and glioblastomas. *International journal of cancer.* Dec 01 2007;121(11):2458-2464.

17. Thon N, Eigenbrod S, Grasbon-Frodl EM, et al. Novel molecular stereotactic biopsy procedures reveal intratumoral homogeneity of loss of heterozygosity of 1p/19q and TP53 mutations in World Health Organization grade II gliomas. *Journal of Neuropathology Experimental Neurology.* Nov 2009;68(11):1219-1228.

18. Van Den Bent MJ, Bromberg JE, Buckner J. Low-grade and anaplastic oligodendroglioma. *Handbook of clinical neurology.* 2016;134:361-380.

19. van den Bent MJ, Brandes AA, Taphoorn MJ, et al. Adjuvant procarbazine, lomustine, and vincristine chemotherapy in newly diagnosed anaplastic oligodendroglioma: long-term follow-up of EORTC brain tumor group study 26951. *Journal of clinical oncology.* Jan 20 2013;31(3):344-350.

20. Cairncross G, Wang M, Shaw E, et al. Phase III trial of chemoradiotherapy for anaplastic oligodendroglioma: long-term results of RTOG 9402. *Journal of clinical oncology.* Jan 20 2013;31(3):337-343.

21. Jansen NL, Graute V, Armbruster L, et al. MRI-suspected low-grade glioma: is there a need to perform dynamic FET PET? *European Journal of Nuclear Medicine and Molecular Imaging.* Jun 2012;39(6):1021-1029.

22. Albert NL, Weller M, Suchorska B, et al. Response Assessment in Neuro-Oncology working group and European Association for Neuro-Oncology recommendations for the clinical use of PET imaging in gliomas. *Neuro-oncology.* Sep 2016;18(9):1199-1208.

23. Langen KJ, Hamacher K, Weckesser M, et al. O-(2-[18F]fluoroethyl)-L-tyrosine: uptake mechanisms and clinical applications. *Nuclear medicine and biology.* Apr 2006;33(3):287-294.

24. Kobayashi K, Ohnishi A, Promsuk J, et al. Enhanced tumor growth elicited by L-type amino acid transporter 1 in human malignant glioma cells. *Neurosurgery.* Feb 2008;62(2):493-503; discussion 503-494.

25. Kickingereder P, Sahm F, Radbruch A, et al. IDH mutation status is associated with a distinct hypoxia/angiogenesis transcriptome signature which is non-invasively predictable with rCBV imaging in human glioma. *Scientific reports.* Nov 05 2015;5:16238.

26. Rapp M, Heinzel A, Galldiks N, et al. Diagnostic performance of 18F-FET PET in newly diagnosed cerebral lesions suggestive of glioma.*Journal of Nuclear Medicine.* Feb 2013;54(2):229-235.

27. Langen KJ, Galldiks N, Hattingen E, Shah NJ. Advances in neuro-oncology imaging. *Nature Reviews Neurology.* May 2017;13(5):279-289.

Figures:

Figure 1. Correlation of Progression Free and Overall Survival Times with the in-group TTP_{min} median within the *IDH 1/2* mut/1p/19q co-del (**A**/**B**) and the *IDH 1/2* mut/1p/19q non-codel (**C**/**D)** group

Figure 2: Correlation of Progression Free and Overall Survival Times with the in-group TTP_{min} median within the *IDH 1/2* wt (**A**/**B**) and the GBM (**C**/**D)** group

Figure 3. Overall Survival times according to WHO 2007 classification (**A**), according to median TBR_{max} values within the WHO grade II and III groups (**B**) and to median TTP_{min} values within the WHO grade II und III groups (**C**) within the *IDH 1/2* mut/1p/19q non-codel group.

Supplemental Figure 1: Overall Survival by age ≤48 years vs. >48 years (**A**); gender (**B**); WHO grade (**C**), *IDH1/2* mutation status (**D**) and IDH1/2 mutation and 1p/19q status and GBM histology (**E**)

Supplemental Figure 2: Example of a patient with an (astrocytic) *IDH1/2* mut/no 1p/19q co-del WHO III tumor with a long time-to-peak time (TTP_{min} >25) minutes and a favorable outcome; following resection and chemotherapy, the patient is still alive after 69 months without further therapy (**A**) In contrast, an example of a patient with an (astrocytic) *IDH1/2* mut/no 1p/19q co-del WHO II tumor and a short time-to-peak (TTP_{min}≤ 25 min) who died after 73 months following biopsy, chemotherapy, as well as multiple salvage-therapies for progressive disease (**B**).

Table

Table 1: Patient characteristics

Factor	Number
Patients	300
Gender (m, f)	166/134
Age, years (median, range)	47.6 (8.1-84.0)
KPS (median, range)	90 (60-100)
Surgical procedure	
Biopsy	238
Surgery	62
WHO grade	
II	121
III	106
IV	73
Tumor location	
Frontal	87
Temporal	129
Parietal	42
Occipital	9
Midline/Basal ganglia/Corpus callosum	33
Eloquent brain area involved	
Yes	170
No	130
Contrast enhancement	
Yes	172
No	128
T_2 volume (mean, median), ml	71.0; 49.0
T_2 diameter (mean, median), cm	5.9; 5.5
CE diameter (mean, median), cm	1.5; 0.8
Molecular markers	
IDH1/2 mut/co-del 1p/19q	58
IDH1/2 mut/no co-del 1p/19q	79
IDH1/2 wt (WHO II+III)	76
GBM	73
First line therapy in addition to surgery/biopsy	
Wait-and-see	67
Chemotherapy	87
Radiotherapy	46
Radiochemotherapy	100

Abbreviations: KPS: Karnofsky Performance Score; WHO: World Health Organization,

CE: contrast enhancement

Table

Table 2: Univariate analysis of ^{18}F-FET-PET parameters within the molecular subgroups

	Factor	N	PFS (months)	95% CI	Log rank p	OS (months)	95% CI	Log rank p
All (including patients without complete molecular profile)	^{18}F-FET negative	45	50.1 (median)	24.0-76.7	0.01	97.9 (mean)**	83.1-112.7	0.001
	^{18}F-FET positive	255	20.0 (median)	15.9-24.3		46.1 (mean)**	26.6-65.6	
	TBR_{max} ≤2.6	147	50.1 (median)	30.0-70.2	<0.001	89.4 (mean)**	79.9-98.9	<0.001
	TBR_{max} >2.6	153	15.4 (median)	12.4-18.4		34.2 (median)	23.0-45.5	
	TTP_{min} ≤17.5	162*	14.2 (median)	11.1-17.3	<0.001	26.2 (median)	21.6-30.8	<0.001
	TTP_{min} >17.5	88*	74.7 (mean)**	62.7-86.7		116.3 (mean)**	106.4-126.3	
***IDH1/2* mut/ 1p/19q co-del** (n=58)	^{18}F-FET negative	3	26.3 (mean)**	18.3-34.2	0.60	All cases censored	na	0.73
	^{18}F-FET positive	55	92.2 (mean)**	78.3-106.2		All cases censored	na	
	TBR_{max} ≤2.7	29	87.9 (median)	10.5-165.4	0.58	All cases censored	na	0.07
	TBR_{max} >2.7	29	95.7 (mean)**	77.5-113.9		All cases censored	na	
	TTP_{min} ≤25	17*	84.2 (mean)**	61.0-107.5	0.54	127.5 (mean)**	114.5-140.6	0.92
	TTP_{min} >25	38*	98.4 (mean)**	84.0-112.7		131.9 (mean)**	122.4-141.3	
***IDH1/2* mut/1p/19q no co-del** (n=79)	^{18}F-FET negative	28	53.8 (median)	41.3-66.3	0.42	99.2 (mean)**	83.7-115.2	0.65
	^{18}F-FET positive	51	37.1 (median)	27.5-46.6		98.1 (mean)**	111.8-110.6	
	TBR_{max} ≤1.7	40	56.8 (median)	40.8-72.8	0.01	104.2 (mean)**	91.5-116.9	0.18
	TBR_{max} >1.7	39	34.9 (median)	18.0-51.9		92.4 (mean)**	76.8-107.9	
	TTP_{min} ≤25	31*	32.3 (median)	16.4-48.2	0.02	75.1 (median)	58.1-92.0	0.002
	TTP_{min} >25	19*	77.0 (mean)**	56.1-97.9		125.6 (mean)**	115.0-136.2	
***IDH1/2* wt** (n=76)	^{18}F-FET negative	9	35.2 (median)	15.8-33.3	0.09	40.9 (median)	14.7-67.0	0.15
	^{18}F-FET positive	67	12.0 (median)	6.5-17.3		24.5 (median)	20.1-28.8	
	TBR_{max} ≤ 2.5	41	14.4 (median)	10.8-17.9	0.52	26.2 (median)	20.7-31.6	0.79
	TBR_{max} >2.5	35	10.4 (median)	1.6-19.1		24.0 (median)	17.4-30.6	
	TTP_{min} ≤12.5	44*	10.4 (median)	4.9-19.9	0.51	24.0 (median)	20.2-27.9	0.14
	TTP_{min} >12.5	23*	11.9 (median)	6.3-17.6		26.0 (median)	15.8-36.1	
GBM (n= 73)	^{18}F-FET negative	0	Not applicable	NA	NA	Not applicable	NA	NA
	^{18}F-FET positive	73	10.3 (median)	9.1-11.5		14.0 (median)	10.4-17.7	
	TBR_{max} ≤ 3.6	36	11.9 (median)	7.6-16.1	0.18	15.8 (median)	7.1-24.5	0.67

	TBR_{max} >3.6	37	8.7 (median)	6.0-11.4		13.4 (median)	10.4-16.3	
	TTP_{min} ≤12.5	49*	9.9 (median)	7.8-12.0	0.36	12.8 (median)	11.2-14.3	0.29
	TTP_{min} >12.5	20*	11.9 (median)	9.1-14.6		23.1 (median)	11.7-34.6	

* TTP was calculated in ^{18}F-FET positive patients only (independent of TBR_{max}); ** median times were not reached; na: not applicable

Table

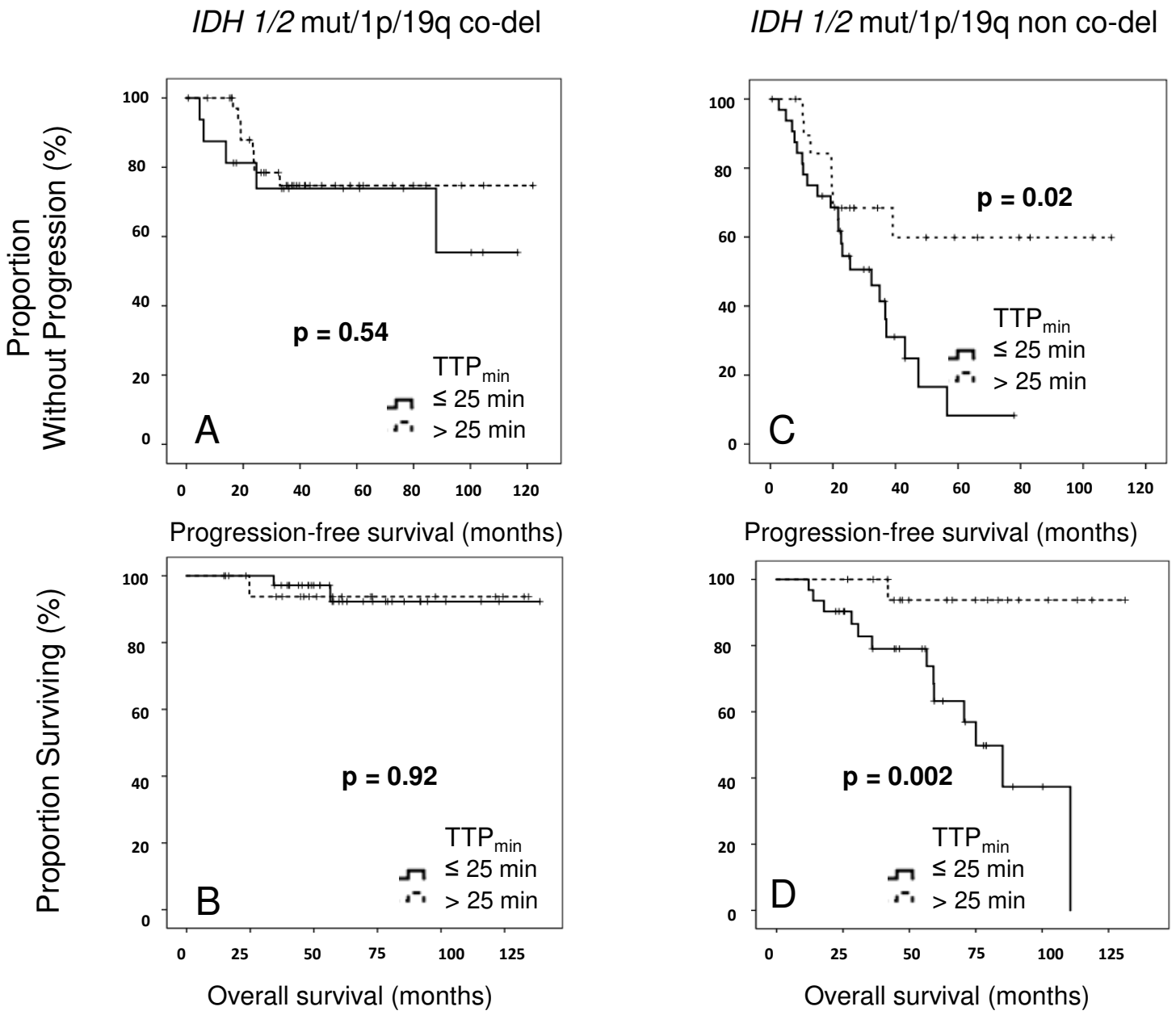
Table 3: Univariate analysis and multivariate analysis

Univariate analysis						
Factor	PFS			OS		
	P value	**HR**	**95%CI HR**	**P value**	**HR**	**95% CI HR**
Age **≤ 48 years** **> 48 years**	<0.001	0.41	0.31-0.56	<0.001	0.27	0.19-0.38
KPS **≥ 90** **< 90**	0.03	0.71	0.52-0.97	<0.001	0.46	0.33-0.64
Surgical procedure[a]	0.36	0.85	0.60-1.21	0.54	0.88	0.60-1.31
Adjuvant therapy[b]	<0.001	0.62	0.54-0.71	<0.001	0.49	0.42-0.58
WHO grade[c]	<0.001	0.44	0.36-0.53	<0.001	0.31	0.25-0.39
***IDH 1/2* mutation**[d]	<0.001	0.17	0.12-0.23	<0.001	0.09	0.06-0.14
FET-negative vs positive	0.01	0.58	0.38-0.89	0.001	0.39	0.22-0.69
TBR_{max} **≤ 2.6** **> 2.6**	<0.001	0.47	0.35-0.64	<0.001	0.47	0.34-0.66
TTP_{min} **>17.5 min** **≤17.5 min**	<0.001	0.29	0.20-0.42	<0.001	0.14	0.08-0.24
CE **no** **yes**	<0.001	0.45	0.33-0.63	<0.001	0.34	0.23-0.48
T_2-volume **≤ 49 ml** **> 49 ml**	0.10	0.78	0.58-1.04	0.39	0.87	0.62-0.83

Multivariate analysis						
	PFS			OS		
Factor	**p-value**	**HR**	**95% CI HR**	**p-value**	**HR**	**95% CI HR**
Age **≤ 48 years** **> 48 years**	0.80	0.95	0.65-1.40	0.11	0.71	0.47-1.07
KPS **≥ 90** **< 90**	0.49	0.94	0.78-1.12	0.75	0.95	0.69-1.31
Adjuvant Therapy[b]	0.23	0.85	0.66-1.11	0.24	0.87	0.61-1.13
WHO grade[c]	0.04	0.65	0.44-0.97	0.007	0.52	0.33-0.84
***IDH 1/2* mutation**[d]	<0.001	0.24	1.15-0.40	<0.001	0.19	0.11-0.34
TBR_{max} **≤ 2.6** **> 2.6**	0.08	0.70	0.47-1.05	0.72	0.93	0.59-1.43
TTP_{min} **> 17.5 min** **≤ 17.5 min**	0.19	0.72	0.44-1.18	0.01	0.43	0.22-0.82
CE **no** **yes**	0.83	0.95	0.61-1.49	0.22	0.71	0.42-1.22

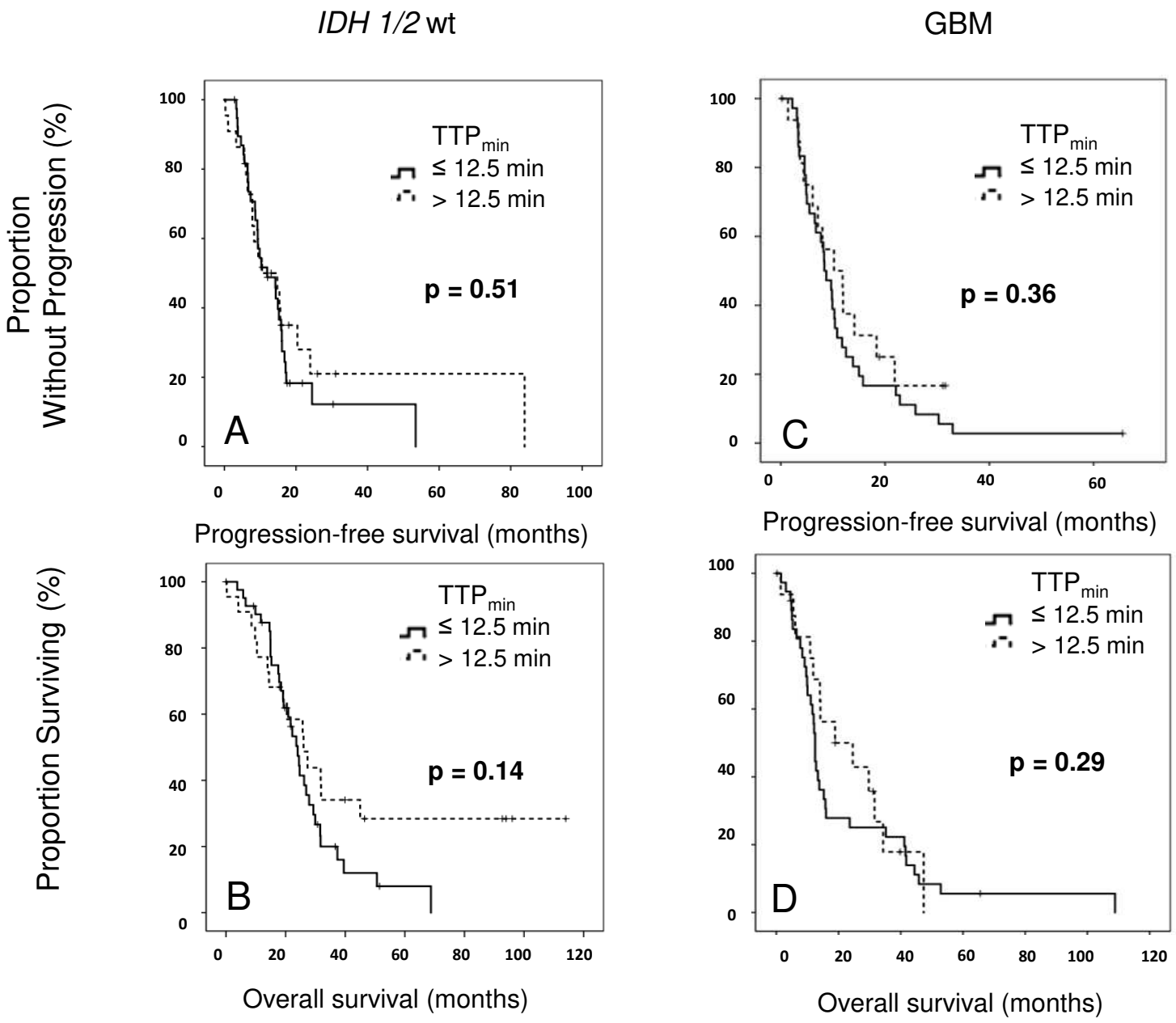
a: surgery vs. biopsy; b: adjuvant therapy: wait-and-see vs. chemotherapy vs. radiation vs. radiochemotherapy; c: WHO grade II vs. III vs. IV; d: *IDH1/2* mut vs. *IDH* wt Abbreviations: OS: Overall Survival; PFS: Progression Free Survival; KPS: Karnofsky Performance Score; WHO: World Health Organization, IDH: isocitrate dehydrogenase; TBR_{max}: maximal tumor-to-brain ratio, TTP_{min}: minimal time-to-peak; CE: contrast enhancement

Figure 1


IDH 1/2 mut/1p/19q co-del
IDH 1/2 mut/1p/19q non co-del
Proportion Without Progression (%)
p = 0.54
TTP$_{min}$
≤ 25 min
> 25 min
A
p = 0.02
C
Progression-free survival (months)
Proportion Surviving (%)
p = 0.92
B
p = 0.002
D
Overall survival (months)

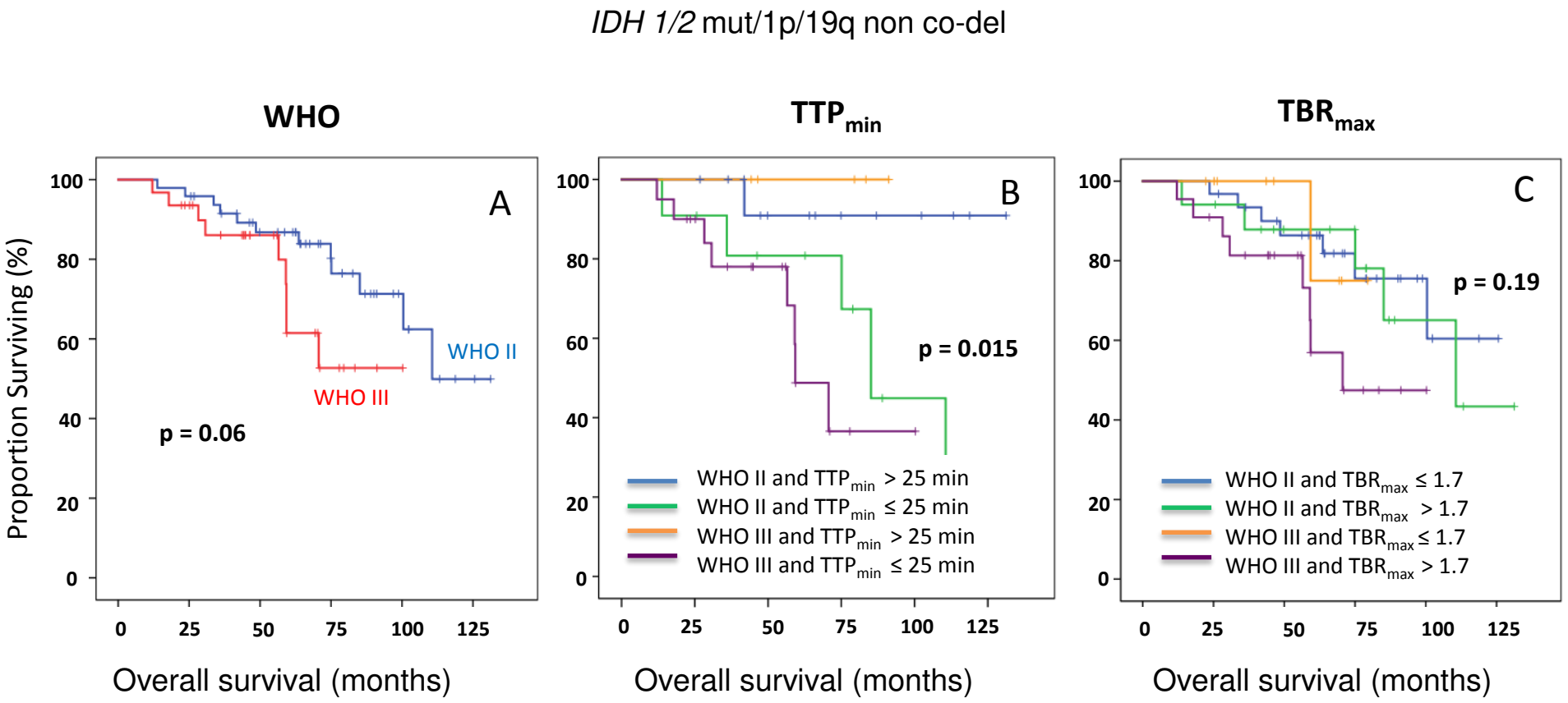
Figure

Figure 2



Figure

Figure 3



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Supplemental Table 1: Median PFS and OS according to WHO grade (2007) and *IDH1/2* mutation/1p/19q co-del status

	PFS (months)	95% CI for PFS	p-value	OS (months)	95% CI for OS	p-value
WHO						
II	87.9	50.1-125.7		110.7 (mean*)	102.4-119.0	
III	19.1	13.2-25.4	<0.001	36.1	16.3-55.9	<0.001
IV	10.3	9.1-11.5		14.0	10.4-17.7	
OA/OD WHO II	85.2 (mean*)	72.3 -98.0		122.8 (mean*)	113.8-131.8	
Diffuse astrocytoma WHO II	53.8	29.9-77.7		102.1	90.9-113.3	
AOA/AOD WHO III	24.6	9.4-39.9	<0.001	56.5	32-6-80.4	<0.001
Anaplastic astrocytoma WHO III	19.3	12.4-26.1		31.8	15.6-48.1	
GBM WHO IV	10.3	9.1-11.5		14.0	10.4-17.7	
***IDH1/2* mut/1p/19q co-del**	91.6 (mean)	78.0-105.3		132. 0 (mean*)	124.5-139.6	
***IDH1/2* mut/1p/19q non co-del**	43.0	27.5-58.6		99.3 (mean*)	88.4-110.6	
***IDH1/2* wt**	14.2	9.5-18.9	<0.001	25.7	22.2-29.2	<0.001
GBM	10.3	9.1-11.5		14.0	10.4-17.7	

*Median not reached; PFS: Progression-free survival; CI: Confidence interval; OS: Overall survival; WHO: World Health Organization; OA: Oligoastrocytoma; OD: Oligodendroglioma; AOA: Anaplastic oligoastrocytoma; AOD: Anaplastic oligodendroglioma; GBM: Glioblastoma; *IDH1/2* mut: Isocitrate dehydrogenase 1/2 mutation; 1p/19q co-del: co-deletion of chromosome arms 1p and 19q; wt: wildtype

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Supplemental Table 2: Detailed patient data

Pat.No	Sex	Age (years)	WHO grade	KPS	*IDH* mut	1p/19q co-deletion	Resection/ Biopsy	Adjuvant therapy	Status	Progression	^{18}F-FET uptake	TBR_{max}	TTP_{min} (minutes)	T_2 - volume (ml)	Contrast enhancement
1	m	71.04	III	80	no	no	Biopsy	radiotherapy	dead	yes	positive	>2.6	<=17.5	>49	yes
2	m	26.90	III	80	yes	no	Resection	chemotherapy	alive	yes	positive	>2.6	<=17.5	>49	yes
3	f	57.37	IV	80	no	no	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes
4	m	36.55	III	70	yes	no	Resection	chemotherapy	alive	no	positive	>2.6	<=17.5	<=49	yes
5	f	57.79	II	90	no	no	Biopsy	radiotherapy	alive	no	positive	>2.6	<=17.5	<=49	yes
6	m	43.10	III	90	yes	no	Resection	radiochemo	alive	yes	positive	>2.6	>17.5	>49	yes
7	m	74.64	II	90	yes	no	Biopsy	wait and see	dead	yes	positive	<=2.6	<=17.5	>49	no
8	f	40.97	II	80	yes	no	Biopsy	chemotherapy	dead	yes	positive	>2.6	<=17.5	>49	yes
9	f	35.00	II	90	yes	yes	Biopsy	chemotherapy	alive	yes	positive	>2.6	<=17.5	<=49	no
10	m	37.36	II	90	yes	yes	Biopsy	wait and see	alive	yes	positive	<=2.6	>17.5	<=49	no
11	f	37.24	III	90	na	na	Biopsy	radiochemo	alive	no	positive	<=2.6	>17.5	>49	yes
12	m	52.88	IV	80	no	no	Resection	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes
13	m	33.16	II	90	no	no	Biopsy	wait and see	alive	no	negative	<=2.6	negative	<=49	no
14	m	26.23	III	90	yes	no	Biopsy	chemotherapy	dead	yes	positive	>2.6	>17.5	>49	yes
15	f	32.82	II	90	no	no	Biopsy	wait and see	alive	no	positive	>2.6	<=17.5	<=49	yes
16	f	52.84	III	90	no	no	Biopsy	chemotherapy	dead	yes	positive	>2.6	<=17.5	<=49	yes
17	f	39.00	IV	90	yes	no	Biopsy	radiochemo	dead	yes	positive	>2.6	>17.5	>49	yes
18	f	46.54	III	90	yes	no	Resection	chemotherapy	alive	no	positive	<=2.6	>17.5	>49	yes
19	m	32.19	II	90	yes	no	Biopsy	chemotherapy	dead	yes	positive	<=2.6	>17.5	<=49	yes
20	f	32.47	II	100	yes	no	Biopsy	wait and see	alive	yes	positive	<=2.6	>17.5	<=49	no
21	f	67.45	IV	90	no	no	Resection	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes
22	f	51.89	II	90	yes	yes	Biopsy	chemotherapy	alive	no	positive	<=2.6	>17.5	<=49	no
23	m	40.56	IV	90	no	no	Biopsy	radiochemo	alive	no	positive	<=2.6	<=17.5	>49	yes
24	m	49.27	II	90	yes	no	Biopsy	chemotherapy	alive	yes	positive	>2.6	>17.5	>49	yes
25	f	45.02	III	80	yes	no	Biopsy	chemotherapy	dead	yes	positive	>2.6	<=17.5	<=49	yes
26	f	41.72	II	90	yes	yes	Biopsy	wait and see	alive	no	positive	<=2.6	>17.5	<=49	no
27	f	74.54	II	80	no	no	Resection	wait and see	dead	yes	positive	>2.6	<=17.5	<=49	yes
28	m	44.22	III	90	yes	no	Biopsy	chemotherapy	dead	yes	positive	>2.6	<=17.5	>49	yes
29	f	47.98	III	80	no	no	Biopsy	chemotherapy	dead	no	positive	>2.6	<=17.5	<=49	yes
30	f	47.77	II	90	no	no	Biopsy	chemotherapy	alive	yes	positive	>2.6	>17.5	>49	yes
31	m	39.28	II	90	yes	yes	Biopsy	chemotherapy	alive	yes	positive	<=2.6	>17.5	>49	no
32	f	45.32	III	80	no	no	Biopsy	chemotherapy	dead	yes	positive	<=2.6	<=17.5	<=49	yes
33	f	48.10	II	80	yes	yes	Biopsy	chemotherapy	alive	no	positive	>2.6	>17.5	>49	no
34	m	70.73	IV	80	no	no	Resection	radiochemo	dead	yes	positive	>2.6	na	>49	yes
35	m	45.26	III	90	no	no	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	<=49	yes
36	m	42.48	III	90	yes	no	Biopsy	radiotherapy	dead	yes	positive	>2.6	<=17.5	>49	yes
37	f	69.14	II	80	yes	no	Biopsy	wait and see	alive	no	positive	<=2.6	na	<=49	no

38	m	51.31	III	90	no	no	Biopsy	radiotherapy	dead	yes	positive	<=2.6	<=17.5	<=49	no
39	m	66.23	IV	80	no	no	Biopsy	radiochemo	alive	no	positive	<=2.6	<=17.5	<=49	yes
40	m	58.55	IV	80	no	no	Resection	radiochemo	alive	no	positive	>2.6	<=17.5	>49	yes
41	m	52.07	IV	80	na	na	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	<=49	yes
42	m	72.56	IV	80	no	no	Biopsy	radiochemo	alive	yes	positive	>2.6	<=17.5	<=49	yes
43	m	33.49	IV	80	no	no	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes
44	f	70.04	II	80	yes	yes	Biopsy	chemotherapy	alive	no	positive	>2.6	<=17.5	>49	yes
45	f	60.11	IV	70	no	no	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	<=49	yes
46	f	63.93	IV	80	no	no	Resection	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes
47	m	48.08	II	90	yes	yes	Biopsy	wait and see	alive	no	positive	>2.6	<=17.5	>49	no
48	m	40.99	II	90	yes	no	Biopsy	chemotherapy	alive	no	positive	<=2.6	>17.5	<=49	no
49	f	49.85	III	80	no	no	Resection	radiochemo	dead	na	positive	>2.6	<=17.5	<=49	yes
50	m	56.45	III	80	no	no	Biopsy	wait and see	alive	na	positive	<=2.6	<=17.5	<=49	no
51	m	50.73	III	90	yes	yes	Biopsy	chemotherapy	alive	no	positive	>2.6	<=17.5	>49	yes
52	f	25.62	III	90	yes	no	Resection	radiotherapy	alive	no	positive	>2.6	>17.5	<=49	yes
53	m	59.78	III	90	yes	yes	Resection	radiotherapy	dead	yes	positive	>2.6	<=17.5	>49	yes
54	m	40.88	II	90	yes	yes	Biopsy	chemotherapy	alive	no	positive	<=2.6	>17.5	>49	no
55	m	42.75	III	90	yes	no	Biopsy	radiochemo	alive	no	positive	<=2.6	>17.5	<=49	no
56	m	43.90	II	100	yes	yes	Biopsy	wait and see	alive	no	positive	<=2.6	>17.5	<=49	yes
57	m	9.08	III	90	no	no	Biopsy	radiotherapy	dead	yes	positive	>2.6	<=17.5	>49	yes
58	f	29.93	III	90	yes	no	Resection	chemotherapy	alive	yes	negative	<=2.6	negative	>49	no
59	m	41.89	IV	90	no	no	Biopsy	wait and see	dead	yes	positive	<=2.6	na	>49	yes
60	f	63.41	IV	80	no	no	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes
61	m	41.58	III	100	yes	no	Biopsy	chemotherapy	alive	no	positive	<=2.6	>17.5	>49	yes
62	m	18.12	II	90	yes	no	Biopsy	wait and see	alive	no	positive	<=2.6	>17.5	<=49	yes
63	f	29.97	II	90	yes	yes	Resection	wait and see	alive	yes	positive	<=2.6	>17.5	<=49	yes
64	m	76.05	IV	90	no	no	Biopsy	radiochemo	alive	na	positive	>2.6	<=17.5	<=49	yes
65	m	39.06	III	90	no	no	Biopsy	chemotherapy	alive	no	positive	<=2.6	<=17.5	<=49	no
66	m	38.28	II	90	yes	no	Biopsy	wait and see	dead	yes	positive	>2.6	<=17.5	>49	no
67	m	62.40	IV	90	no	no	Resection	radiochemo	dead	yes	positive	<=2.6	>17.5	>49	yes
68	m	42.09	II	80	yes	no	Biopsy	chemotherapy	alive	no	positive	<=2.6	>17.5	<=49	no
69	m	27.18	II	90	yes	no	Resection	wait and see	dead	yes	negative	<=2.6	negative	<=49	no
70	f	62.54	II	90	no	no	Biopsy	wait and see	alive	no	negative	<=2.6	negative	>49	yes
71	m	31.77	III	90	yes	yes	Biopsy	radiochemo	alive	yes	positive	>2.6	<=17.5	>49	yes
72	m	41.81	II	90	yes	no	Biopsy	chemotherapy	alive	no	positive	>2.6	<=17.5	<=49	no
73	m	46.98	III	90	no	no	Biopsy	radiotherapy	dead	yes	positive	>2.6	>17.5	<=49	yes
74	f	43.41	III	90	no	no	Biopsy	radiotherapy	alive	na	positive	>2.6	<=17.5	<=49	no
75	m	52.24	II	90	yes	yes	Biopsy	chemotherapy	alive	no	positive	>2.6	<=17.5	>49	yes
76	m	62.03	IV	80	na	na	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	<=49	yes
77	m	50.71	III	90	no	no	Resection	radiochemo	dead	yes	positive	<=2.6	<=17.5	>49	yes
78	f	49.34	II	90	yes	no	Biopsy	wait and see	alive	no	negative	<=2.6	negative	<=49	no
79	m	29.07	II	90	yes	no	Resection	wait and see	alive	yes	negative	<=2.6	negative	<=49	no
80	m	58.81	II	90	yes	yes	Biopsy	wait and see	alive	no	positive	<=2.6	>17.5	<=49	no

81	f	41.79	II	70	na	na	Biopsy	radiotherapy	alive	no	positive	<=2.6	>17.5	<=49	no
82	m	49.47	IV	70	no	no	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	<=49	yes
83	m	67.06	IV	70	no	no	Resection	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes
84	f	60.38	IV	80	no	no	Biopsy	wait and see	dead	yes	positive	>2.6	<=17.5	<=49	no
85	m	34.84	III	90	no	no	Resection	radiochemo	dead	yes	positive	<=2.6	>17.5	<=49	yes
86	f	76.68	II	90	no	no	Biopsy	radiochemo	dead	no	positive	>2.6	>17.5	>49	yes
87	m	34.87	II	90	yes	yes	Resection	wait and see	alive	no	positive	>2.6	>17.5	<=49	no
88	m	67.92	II	90	no	no	Biopsy	radiotherapy	dead	yes	negative	<=2.6	negative	<=49	no
89	m	35.47	II	90	yes	no	Biopsy	radiotherapy	dead	yes	negative	<=2.6	negative	<=49	no
90	f	45.36	II	90	yes	yes	Biopsy	radiotherapy	alive	no	positive	<=2.6	>17.5	<=49	no
91	m	30.49	II	90	yes	yes	Biopsy	wait and see	alive	no	positive	<=2.6	>17.5	<=49	yes
92	m	43.08	II	90	yes	yes	Biopsy	wait and see	alive	no	negative	<=2.6	negative	>49	no
93	f	51.44	III	90	no	no	Biopsy	radiotherapy	dead	yes	positive	<=2.6	<=17.5	>49	no
94	m	40.59	III	80	no	no	Biopsy	radiochemo	dead	yes	positive	>2.6	>17.5	>49	yes
95	f	57.27	III	90	no	no	Biopsy	chemotherapy	alive	no	positive	<=2.6	<=17.5	<=49	yes
96	m	74.50	IV	90	na	na	Resection	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes
97	m	62.39	III	90	no	no	Biopsy	chemotherapy	alive	yes	positive	>2.6	<=17.5	>49	no
98	f	65.19	IV	70	no	no	Resection	radiochemo	dead	yes	positive	<=2.6	<=17.5	>49	yes
99	f	21.75	III	90	yes	no	Resection	chemotherapy	alive	na	negative	<=2.6	negative	<=49	yes
100	f	64.97	IV	90	no	no	Biopsy	radiochemo	dead	yes	positive	>2.6	>17.5	>49	yes
101	m	73.70	IV	70	no	no	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes
102	f	45.51	II	90	yes	yes	Biopsy	wait and see	alive	yes	positive	>2.6	>17.5	>49	no
103	m	42.95	II	90	no	no	Biopsy	radiotherapy	dead	yes	positive	<=2.6	>17.5	<=49	no
104	m	60.13	III	90	no	no	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	<=49	yes
105	f	46.30	II	80	yes	yes	Biopsy	chemotherapy	alive	no	positive	>2.6	>17.5	>49	yes
106	m	28.02	II	90	yes	no	Biopsy	wait and see	alive	no	negative	<=2.6	negative	>49	no
110	m	67.33	IV	80	no	no	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	<=49	yes
111	f	61.59	III	70	no	no	Biopsy	radiochemo	alive	no	positive	>2.6	>17.5	<=49	no
112	m	37.15	II	90	yes	yes	Resection	radiotherapy	alive	no	positive	>2.6	>17.5	>49	yes
113	m	39.67	IV	80	no	no	Resection	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes
114	m	74.18	IV	80	no	no	Resection	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes
115	f	49.58	II	90	yes	yes	Biopsy	wait and see	alive	no	positive	<=2.6	>17.5	>49	no
116	m	47.54	II	90	yes	no	Biopsy	wait and see	dead	no	negative	<=2.6	negative	>49	no
117	f	40.60	II	90	yes	yes	Biopsy	wait and see	alive	no	positive	<=2.6	>17.5	<=49	no
118	m	55.23	IV	60	no	no	Resection	radiochemo	dead	yes	positive	<=2.6	na	>49	yes
119	f	53.57	IV	100	no	no	Resection	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes
120	m	64.31	II	90	na	na	Biopsy	radiotherapy	dead	yes	negative	<=2.6	negative	<=49	no
121	m	65.12	IV	90	no	no	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	<=49	yes
122	f	57.80	II	90	yes	yes	Resection	wait and see	alive	no	positive	>2.6	<=17.5	>49	yes
123	m	50.36	II	90	no	no	Biopsy	wait and see	alive	yes	positive	<=2.6	>17.5	<=49	yes
124	f	48.36	II	80	yes	yes	Biopsy	chemotherapy	alive	no	positive	>2.6	>17.5	<=49	yes
125	m	54.07	III	80	no	no	Biopsy	radiochemo	dead	no	positive	<=2.6	<=17.5	>49	no
126	f	52.90	IV	90	na	na	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes

127	f	45.80	II	100	yes	no	Resection	wait and see	alive	yes	negative	<=2.6	negative	<=49	no
128	m	19.00	III	70	no	no	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	<=49	yes
129	m	45.96	II	90	na	na	Biopsy	wait and see	dead	yes	positive	<=2.6	>17.5	<=49	no
130	m	38.30	II	90	yes	yes	Resection	wait and see	alive	no	positive	>2.6	<=17.5	>49	no
131	m	67.35	IV	90	no	no	Biopsy	radiochemo	alive	na	positive	>2.6	<=17.5	>49	yes
132	m	25.88	III	90	yes	no	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	<=49	yes
133	f	38.46	II	90	yes	no	Biopsy	chemotherapy	alive	yes	positive	<=2.6	>17.5	<=49	yes
134	f	69.50	IV	90	no	no	Resection	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes
135	m	46.51	IV	80	no	no	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	<=49	yes
136	f	8.07	III	80	no	no	Biopsy	chemotherapy	alive	na	positive	<=2.6	<=17.5	<=49	no
137	f	26.68	II	90	yes	yes	Biopsy	wait and see	alive	no	negative	<=2.6	negative	<=49	no
138	f	68.85	IV	90	no	no	Biopsy	radiochemo	dead	yes	positive	<=2.6	<=17.5	>49	yes
139	f	63.99	IV	90	no	no	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes
140	m	28.62	III	100	yes	yes	Resection	chemotherapy	alive	no	positive	<=2.6	>17.5	<=49	yes
141	m	75.92	II	90	no	no	Biopsy	wait and see	dead	yes	positive	<=2.6	<=17.5	<=49	no
142	m	73.07	III	90	no	no	Biopsy	radiochemo	alive	no	positive	>2.6	<=17.5	>49	yes
143	f	37.39	III	90	no	no	Biopsy	radiochemo	alive	yes	positive	>2.6	<=17.5	<=49	no
144	m	43.23	II	100	yes	yes	Biopsy	chemotherapy	alive	no	positive	<=2.6	>17.5	<=49	no
145	m	25.86	III	100	no	no	Biopsy	radiochemo	alive	na	positive	>2.6	<=17.5	>49	yes
146	f	47.62	IV	80	no	no	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes
147	f	38.22	IV	80	na	na	Biopsy	radiochemo	alive	no	positive	>2.6	<=17.5	>49	yes
148	f	34.70	II	90	yes	no	Biopsy	chemotherapy	dead	yes	positive	<=2.6	>17.5	>49	no
149	m	58.21	IV	70	no	no	Resection	radiochemo	dead	yes	positive	>2.6	<=17.5	<=49	yes
150	m	54.91	II	90	no	no	Biopsy	wait and see	dead	yes	negative	<=2.6	negative	>49	no
151	m	66.68	III	70	no	no	Biopsy	radiotherapy	dead	yes	positive	<=2.6	<=17.5	<=49	no
152	m	63.45	IV	80	no	no	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	<=49	yes
153	m	40.48	IV	90	yes	yes	Resection	radiochemo	alive	no	positive	>2.6	<=17.5	>49	yes
154	m	58.95	III	70	no	no	Biopsy	radiochemo	alive	yes	positive	>2.6	<=17.5	>49	yes
155	f	41.68	II	90	yes	no	Biopsy	wait and see	alive	no	negative	<=2.6	negative	<=49	no
156	f	35.04	III	90	na	na	Resection	radiotherapy	alive	no	negative	<=2.6	negative	<=49	no
157	m	27.76	II	90	yes	no	Biopsy	wait and see	alive	yes	positive	<=2.6	>17.5	<=49	no
158	f	61.53	IV	80	no	no	Resection	radiochemo	dead	no	positive	>2.6	<=17.5	<=49	yes
159	m	37.33	II	90	yes	no	Biopsy	radiotherapy	alive	no	positive	<=2.6	<=17.5	<=49	no
160	m	42.09	II	100	yes	yes	Biopsy	chemotherapy	alive	no	positive	<=2.6	<=17.5	>49	no
161	f	31.14	III	90	yes	no	Biopsy	radiotherapy	dead	yes	positive	<=2.6	>17.5	<=49	yes
162	f	56.42	III	90	no	no	Biopsy	chemotherapy	dead	yes	positive	<=2.6	<=17.5	<=49	yes
163	f	51.18	III	80	yes	yes	Biopsy	chemotherapy	alive	no	positive	>2.6	>17.5	>49	no
164	m	45.23	II	90	no	no	Biopsy	radiotherapy	alive	no	negative	<=2.6	negative	<=49	no
165	m	33.01	II	90	yes	yes	Biopsy	chemotherapy	alive	no	positive	<=2.6	<=17.5	<=49	no
166	m	58.70	III	90	yes	no	Biopsy	chemotherapy	alive	no	positive	<=2.6	<=17.5	>49	yes
167	f	69.54	IV	70	no	no	Resection	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes
168	m	46.90	III	90	yes	no	Biopsy	chemotherapy	alive	no	positive	<=2.6	>17.5	>49	no
169	m	40.39	III	90	yes	no	Resection	chemotherapy	alive	yes	positive	>2.6	>17.5	>49	yes

170	f	81.35	II	100	yes	yes	Biopsy	chemotherapy	alive	no	positive	<=2.6	>17.5	<=49	no
171	f	42.20	II	100	yes	no	Biopsy	radiotherapy	alive	yes	negative	<=2.6	negative	<=49	no
172	m	43.66	III	90	na	na	Biopsy	radiochemo	dead	yes	positive	<=2.6	<=17.5	>49	yes
173	f	38.74	II	90	yes	yes	Biopsy	chemotherapy	alive	no	positive	>2.6	>17.5	<=49	no
174	m	61.10	IV	90	no	no	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes
175	f	39.45	II	100	yes	no	Resection	wait and see	alive	no	negative	<=2.6	negative	<=49	no
176	f	27.43	II	90	yes	yes	Biopsy	wait and see	alive	yes	negative	<=2.6	negative	<=49	no
177	m	66.29	IV	70	no	no	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	<=49	yes
178	m	24.68	II	100	yes	no	Resection	radiotherapy	alive	yes	positive	<=2.6	>17.5	>49	no
179	f	27.00	III	90	yes	no	Biopsy	chemotherapy	alive	yes	negative	<=2.6	negative	>49	no
180	f	36.62	III	90	no	no	Biopsy	chemotherapy	dead	no	positive	>2.6	<=17.5	<=49	yes
181	f	59.62	IV	90	no	no	Biopsy	radiochemo	alive	no	positive	>2.6	<=17.5	<=49	yes
182	m	38.29	II	80	yes	no	Biopsy	wait and see	alive	no	positive	<=2.6	>17.5	<=49	no
183	f	65.83	II	90	na	na	Biopsy	wait and see	dead	yes	positive	<=2.6	<=17.5	>49	no
184	f	30.26	II	90	yes	yes	Biopsy	wait and see	alive	no	positive	<=2.6	>17.5	>49	no
185	m	32.74	II	90	na	na	Biopsy	radiotherapy	alive	yes	negative	<=2.6	negative	<=49	no
186	m	26.06	II	90	yes	yes	Biopsy	radiotherapy	alive	no	positive	<=2.6	>17.5	<=49	no
187	f	45.82	II	90	yes	yes	Biopsy	chemotherapy	alive	no	positive	>2.6	>17.5	<=49	no
188	f	50.23	III	90	no	no	Resection	chemotherapy	dead	yes	positive	<=2.6	<=17.5	>49	no
189	f	57.83	II	90	na	na	Biopsy	wait and see	alive	no	positive	<=2.6	>17.5	<=49	yes
190	m	49.04	IV	90	no	no	Biopsy	chemotherapy	dead	yes	positive	>2.6	<=17.5	<=49	yes
191	m	59.28	III	90	no	no	Biopsy	radiochemo	alive	yes	positive	>2.6	<=17.5	<=49	no
192	f	63.43	II	90	na	na	Biopsy	radiochemo	dead	yes	positive	>2.6	>17.5	<=49	no
193	f	47.77	III	80	no	no	Biopsy	radiotherapy	dead	yes	positive	>2.6	<=17.5	>49	yes
194	f	62.08	IV	80	no	no	Resection	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes
195	m	47.19	III	90	no	no	Biopsy	radiotherapy	dead	yes	positive	<=2.6	<=17.5	<=49	yes
196	m	42.58	III	90	yes	no	Biopsy	chemotherapy	dead	yes	negative	<=2.6	negative	>49	no
197	m	44.17	II	90	yes	no	Biopsy	wait and see	alive	no	negative	<=2.6	negative	<=49	no
198	f	72.67	II	70	no	no	Biopsy	wait and see	dead	no	positive	<=2.6	<=17.5	<=49	no
199	f	30.87	II	90	yes	yes	Biopsy	wait and see	alive	no	positive	<=2.6	>17.5	<=49	yes
200	f	72.30	II	90	no	no	Biopsy	wait and see	alive	yes	negative	<=2.6	negative	>49	no
201	f	35.66	II	90	yes	yes	Biopsy	chemotherapy	alive	yes	positive	>2.6	<=17.5	>49	yes
202	m	71.88	III	70	yes	no	Resection	chemotherapy	alive	no	negative	<=2.6	negative	<=49	yes
203	f	37.64	IV	90	no	no	Biopsy	radiochemo	alive	yes	positive	>2.6	<=17.5	>49	yes
204	m	43.32	III	100	yes	yes	Biopsy	chemotherapy	alive	no	positive	<=2.6	<=17.5	>49	no
205	m	49.17	II	90	yes	no	Biopsy	radiotherapy	dead	yes	negative	<=2.6	negative	<=49	no
206	f	74.75	III	80	no	no	Biopsy	radiotherapy	dead	yes	positive	>2.6	<=17.5	<=49	yes
207	m	75.62	III	80	yes	yes	Resection	chemotherapy	alive	na	positive	>2.6	<=17.5	>49	yes
208	m	58.43	II	90	yes	yes	Biopsy	chemotherapy	alive	yes	positive	>2.6	>17.5	<=49	yes
209	m	66.81	IV	80	no	no	Resection	radiochemo	dead	na	positive	>2.6	<=17.5	>49	yes
210	f	50.43	III	80	no	no	Biopsy	radiochemo	dead	yes	positive	<=2.6	<=17.5	>49	yes
211	m	72.94	IV	80	no	no	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes
212	f	76.34	III	60	no	no	Biopsy	wait and see	dead	yes	positive	<=2.6	<=17.5	>49	no

213	f	54.13	IV	80	no	no	Resection	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes
214	m	37.46	III	70	yes	no	Biopsy	radiotherapy	alive	yes	positive	>2.6	<=17.5	>49	yes
215	m	49.48	III	90	yes	no	Biopsy	chemotherapy	dead	yes	positive	>2.6	>17.5	>49	yes
216	f	63.42	IV	90	na	na	Resection	radiochemo	dead	yes	positive	>2.6	<=17.5	<=49	yes
217	m	39.76	II	80	no	no	Biopsy	radiotherapy	alive	no	negative	<=2.6	negative	>49	no
218	f	71.69	IV	90	no	no	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes
219	m	16.21	II	90	yes	no	Biopsy	wait and see	alive	no	negative	<=2.6	negative	<=49	no
220	m	38.43	III	100	yes	no	Resection	radiotherapy	dead	yes	positive	>2.6	<=17.5	>49	yes
221	m	45.04	III	90	yes	no	Resection	chemotherapy	alive	no	positive	<=2.6	>17.5	<=49	yes
222	f	20.39	III	80	no	no	Biopsy	radiochemo	dead	yes	positive	<=2.6	<=17.5	>49	no
223	f	53.86	II	70	yes	no	Biopsy	wait and see	alive	yes	negative	<=2.6	negative	>49	no
224	f	31.58	III	90	yes	no	Biopsy	chemotherapy	alive	no	positive	<=2.6	<=17.5	>49	no
225	m	70.45	III	90	no	no	Biopsy	chemotherapy	dead	yes	positive	<=2.6	<=17.5	<=49	no
226	m	56.61	III	80	no	no	Biopsy	radiotherapy	dead	yes	negative	<=2.6	negative	<=49	no
227	f	43.52	IV	90	yes	no	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes
228	m	64.48	II	90	no	no	Biopsy	wait and see	alive	no	positive	<=2.6	>17.5	<=49	no
229	f	66.97	III	70	no	no	Biopsy	radiotherapy	dead	yes	positive	>2.6	<=17.5	>49	yes
230	m	18.32	II	90	na	na	Biopsy	radiotherapy	alive	no	negative	<=2.6	negative	<=49	no
231	m	35.75	II	90	no	no	Biopsy	radiotherapy	dead	yes	negative	<=2.6	negative	<=49	no
232	f	61.02	III	90	no	no	Biopsy	radiochemo	dead	yes	positive	<=2.6	<=17.5	<=49	yes
233	m	49.86	IV	80	no	no	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	<=49	yes
234	f	37.29	II	90	yes	no	Biopsy	chemotherapy	alive	yes	negative	<=2.6	negative	>49	no
235	m	54.59	III	90	yes	yes	Biopsy	chemotherapy	alive	no	positive	>2.6	>17.5	>49	no
236	m	50.87	IV	70	no	no	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes
237	f	34.81	II	80	yes	no	Resection	wait and see	alive	yes	positive	>2.6	<=17.5	<=49	yes
238	m	76.87	IV	80	yes	no	Biopsy	radiochemo	alive	yes	positive	>2.6	<=17.5	>49	yes
239	f	15.66	II	90	yes	yes	Biopsy	radiotherapy	alive	no	positive	>2.6	<=17.5	<=49	yes
240	f	70.04	IV	70	no	no	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes
241	f	67.96	III	80	yes	yes	Biopsy	chemotherapy	alive	no	positive	>2.6	<=17.5	>49	yes
242	f	57.18	III	90	na	na	Biopsy	chemotherapy	alive	no	positive	>2.6	>17.5	<=49	no
243	m	56.88	III	90	no	no	Biopsy	radiotherapy	dead	yes	positive	<=2.6	<=17.5	<=49	yes
244	f	77.17	III	80	no	no	Biopsy	radiotherapy	dead	no	positive	>2.6	<=17.5	>49	yes
245	m	51.43	III	90	yes	no	Biopsy	chemotherapy	dead	yes	positive	>2.6	<=17.5	>49	yes
246	m	42.97	II	80	no	no	Biopsy	chemotherapy	alive	no	positive	>2.6	<=17.5	<=49	yes
247	m	68.35	IV	80	no	no	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	<=49	yes
248	m	59.36	IV	90	yes	no	Biopsy	radiochemo	dead	yes	positive	<=2.6	>17.5	>49	no
249	f	35.60	II	70	no	no	Biopsy	chemotherapy	alive	yes	positive	>2.6	>17.5	>49	no
250	f	43.06	II	90	yes	no	Biopsy	wait and see	alive	no	positive	<=2.6	>17.5	<=49	no
251	f	44.31	II	80	yes	yes	Biopsy	chemotherapy	alive	no	positive	<=2.6	>17.5	>49	no
252	m	55.72	III	90	no	no	Biopsy	chemotherapy	dead	yes	positive	>2.6	<=17.5	>49	no
253	f	73.52	III	90	no	no	Biopsy	radiochemo	dead	yes	positive	<=2.6	<=17.5	>49	yes
254	f	68.91	IV	90	no	no	Biopsy	radiochemo	dead	yes	positive	<=2.6	<=17.5	<=49	yes
255	m	62.61	II	90	yes	yes	Biopsy	radiotherapy	alive	no	positive	>2.6	<=17.5	<=49	yes

256	m	60.45	IV	60	no	no	Resection	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes
257	m	66.52	III	70	no	no	Biopsy	chemotherapy	dead	yes	positive	>2.6	<=17.5	<=49	yes
258	f	49.25	III	80	no	no	Biopsy	radiochemo	dead	yes	positive	<=2.6	>17.5	>49	no
259	f	53.21	III	90	yes	yes	Biopsy	chemotherapy	dead	yes	positive	>2.6	>17.5	>49	yes
260	m	59.41	III	90	no	no	Biopsy	chemotherapy	dead	yes	positive	<=2.6	<=17.5	>49	no
261	f	44.86	II	90	yes	no	Biopsy	chemotherapy	alive	no	negative	<=2.6	negative	>49	no
262	f	50.10	II	90	yes	yes	Resection	chemotherapy	alive	no	positive	>2.6	>17.5	>49	yes
263	f	59.20	III	90	no	no	Biopsy	chemotherapy	dead	yes	positive	>2.6	<=17.5	<=49	yes
264	m	47.42	II	70	yes	no	Biopsy	chemotherapy	alive	no	positive	<=2.6	>17.5	>49	no
265	m	66.19	IV	80	no	no	Resection	radiochemo	dead	yes	positive	<=2.6	na	>49	yes
266	f	47.18	IV	90	no	no	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	<=49	yes
267	f	49.06	IV	70	na	na	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	<=49	yes
268	f	44.42	III	90	no	no	Biopsy	radiotherapy	dead	yes	positive	<=2.6	<=17.5	<=49	no
269	f	35.51	II	80	yes	no	Resection	wait and see	alive	yes	negative	<=2.6	negative	>49	no
270	m	53.43	III	90	no	no	Biopsy	chemotherapy	dead	yes	positive	<=2.6	<=17.5	<=49	no
271	f	42.26	II	90	yes	no	Biopsy	wait and see	alive	no	negative	<=2.6	negative	<=49	no
272	f	29.27	III	100	yes	no	Resection	chemotherapy	alive	no	positive	<=2.6	>17.5	<=49	no
273	m	34.25	II	90	yes	no	Biopsy	chemotherapy	alive	no	positive	<=2.6	>17.5	>49	yes
274	f	41.00	III	100	yes	yes	Resection	wait and see	alive	no	positive	>2.6	>17.5	<=49	yes
275	f	28.99	II	90	yes	no	Biopsy	wait and see	alive	no	positive	<=2.6	>17.5	>49	yes
276	f	47.60	II	90	yes	no	Biopsy	wait and see	alive	no	positive	<=2.6	>17.5	>49	no
277	f	55.73	IV	90	na	na	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	<=49	yes
278	m	27.71	II	100	yes	no	Biopsy	wait and see	alive	no	negative	<=2.6	negative	<=49	no
279	m	73.35	III	90	no	no	Biopsy	radiotherapy	dead	yes	positive	>2.6	<=17.5	<=49	yes
280	m	30.93	II	90	yes	no	Biopsy	wait and see	alive	yes	negative	<=2.6	negative	<=49	no
281	m	31.90	II	90	yes	yes	Biopsy	chemotherapy	alive	yes	positive	>2.6	<=17.5	>49	no
282	m	47.03	IV	80	no	no	Biopsy	chemotherapy	dead	yes	positive	>2.6	<=17.5	>49	yes
283	m	49.77	III	90	yes	no	Biopsy	radiochemo	alive	yes	positive	<=2.6	>17.5	>49	no
284	m	24.79	III	70	na	na	Biopsy	radiochemo	dead	yes	negative	<=2.6	negative	<=49	no
285	f	46.10	III	90	no	no	Biopsy	wait and see	alive	no	positive	>2.6	<=17.5	<=49	no
286	m	50.55	III	90	yes	no	Biopsy	radiotherapy	dead	yes	positive	<=2.6	>17.5	<=49	no
287	f	40.39	II	80	yes	yes	Biopsy	wait and see	alive	yes	positive	<=2.6	>17.5	>49	yes
288	f	23.41	II	90	yes	no	Biopsy	radiotherapy	dead	yes	negative	<=2.6	negative	<=49	no
289	m	46.25	II	90	yes	yes	Biopsy	chemotherapy	alive	no	positive	>2.6	>17.5	>49	yes
290	m	61.83	IV	90	no	no	Resection	chemotherapy	dead	yes	positive	>2.6	<=17.5	<=49	yes
291	m	29.65	IV	80	yes	yes	Biopsy	radiochemo	dead	no	positive	<=2.6	<=17.5	<=49	yes
292	m	53.04	III	90	no	no	Biopsy	radiochemo	alive	yes	positive	>2.6	<=17.5	>49	yes
293	m	47.53	II	80	yes	no	Biopsy	radiotherapy	dead	yes	negative	<=2.6	negative	<=49	no
294	f	22.77	II	90	yes	no	Biopsy	wait and see	alive	no	positive	<=2.6	>17.5	<=49	no
295	m	31.06	II	80	yes	yes	Biopsy	chemotherapy	alive	no	positive	>2.6	<=17.5	>49	no
296	m	63.99	III	90	yes	no	Biopsy	chemotherapy	alive	yes	positive	>2.6	>17.5	>49	no
297	f	83.99	III	70	no	no	Biopsy	wait and see	alive	na	positive	<=2.6	<=17.5	>49	no
298	f	28.67	III	90	yes	no	Resection	wait and see	alive	no	negative	<=2.6	negative	<=49	no

299	m	32.55	II	100	yes	yes	Resection	chemotherapy	dead	yes	positive	>2.6	<=17.5	<=49	yes
300	m	47.61	IV	70	na	na	Biopsy	radiochemo	dead	yes	positive	>2.6	<=17.5	>49	yes

Supplementary file

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Supplemental Table 3: Patient characteristics in the *IDH1/2* mut/1p/19q no co-del subgroup according to TTP_{min}

	TTP_{min} ≤25 min	TTP_{min} >25 min	p-value
WHO grade			
II	11	14	0.02
III	20	5	
age (median)	40.3	40.4	0.32*
KPS (median)	90	90	0.74*
Surgical Procedure			
Biopsy	22	16	0.33
Surgery	9	3	
Adjuvant therapy			
Wait-and-see	5	8	0.17
Chemotherapy	18	9	
Radiotherapy	6	1	
Radiochemotherapy	2	1	

Abbreviations: WHO: World Health Organisation; TTP_{min}: minimal time-to-peak; KPS: Karnofsky Performance Score.

* Mann Whitney-U test, all other p-values were calculated using X^2 Test

Supplementary file

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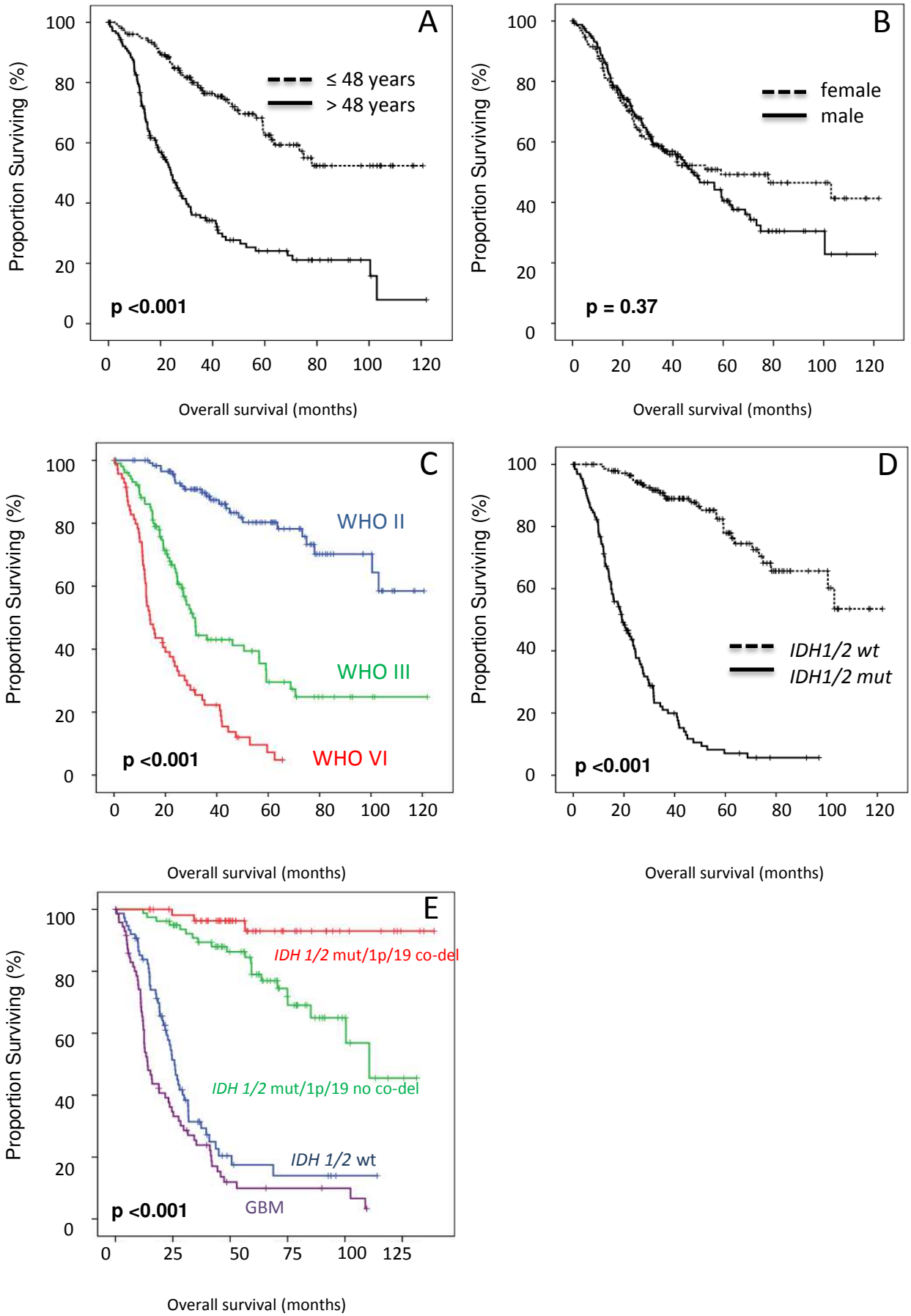
Supplemental Figure 1



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Supplemental Figure 2